Rapid PBPK modeling with the httk model

A Course on Physiologically Based Pharmacokinetic (PBPK) Modeling in the 21st Century

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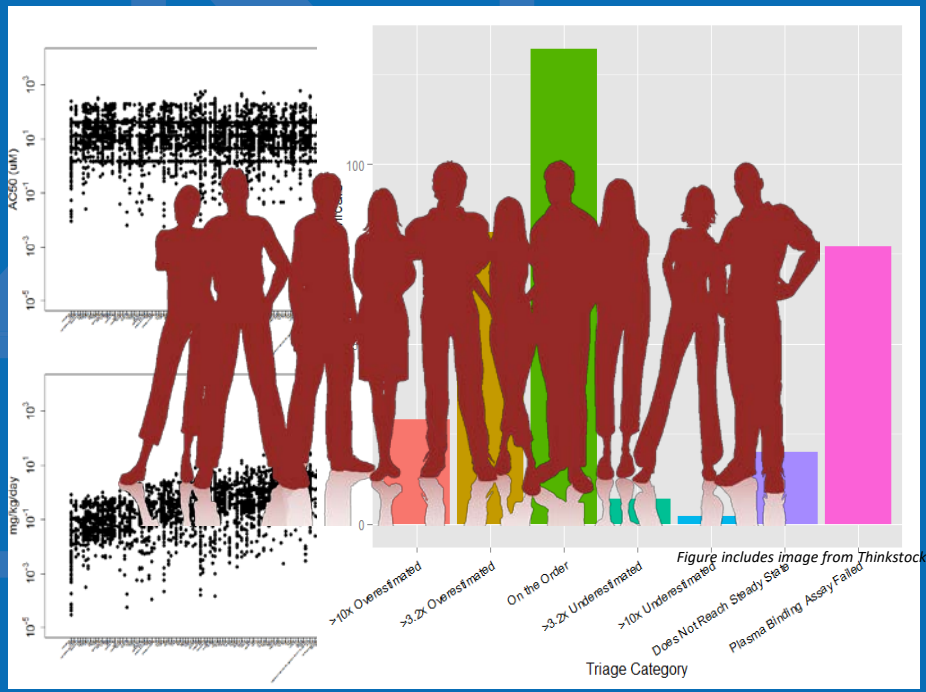


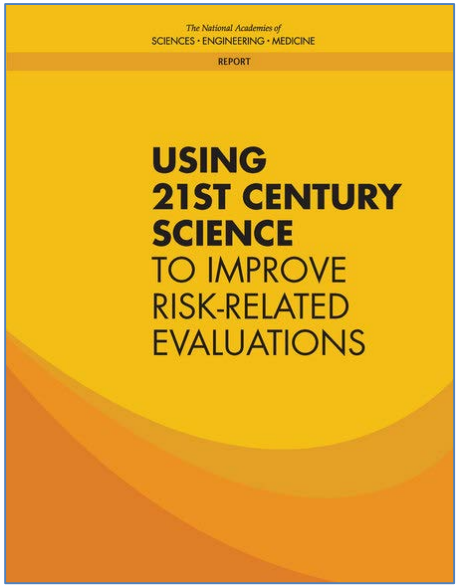
Figure includes image from Thinkstock

orcid.org/0000-0002-4024-534X

High-Throughput Bioactivity Screening

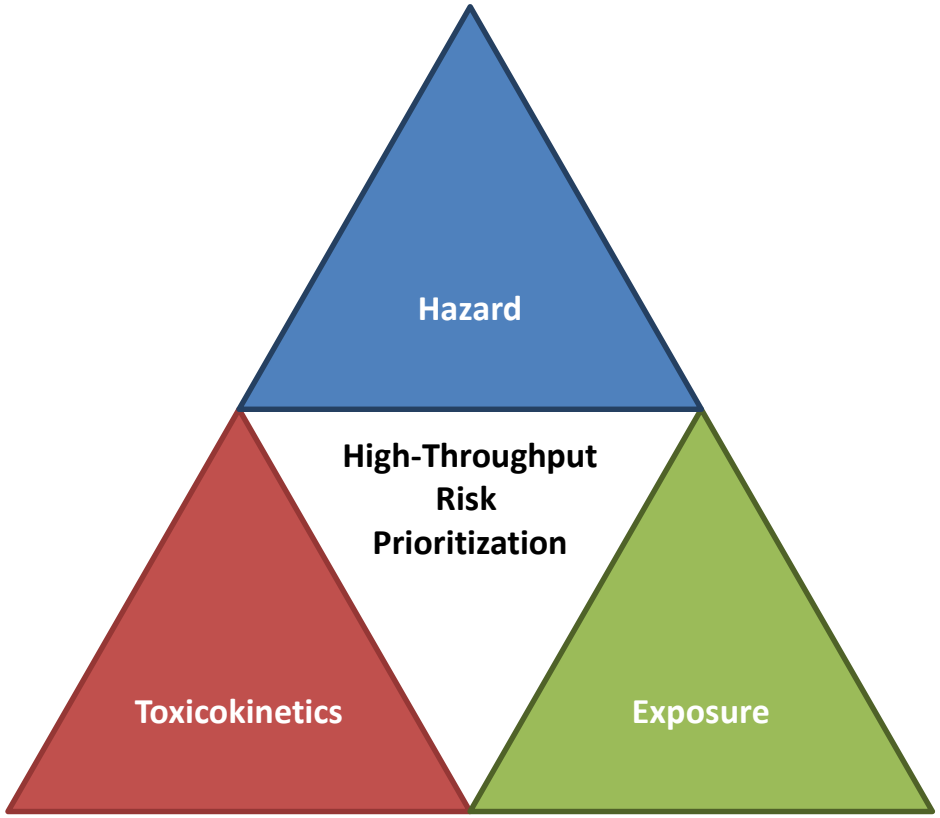
- **Tox21**: Examining >8,000 chemicals using ~50 assays intended to identify interactions with biological pathways (Schmidt, 2009)
- **ToxCast**: For a subset (>2000) of Tox21 chemicals ran >1100 additional assays (Judson *et al.*, 2010)
- Most assays conducted in dose-response format (identify 50% activity concentration – AC50 – and efficacy if data described by a Hill function, Filer *et al.*, 2016)
- Data are public: https://comptox.epa.gov/dashboard
- National Academy of Sciences, January, 2017:

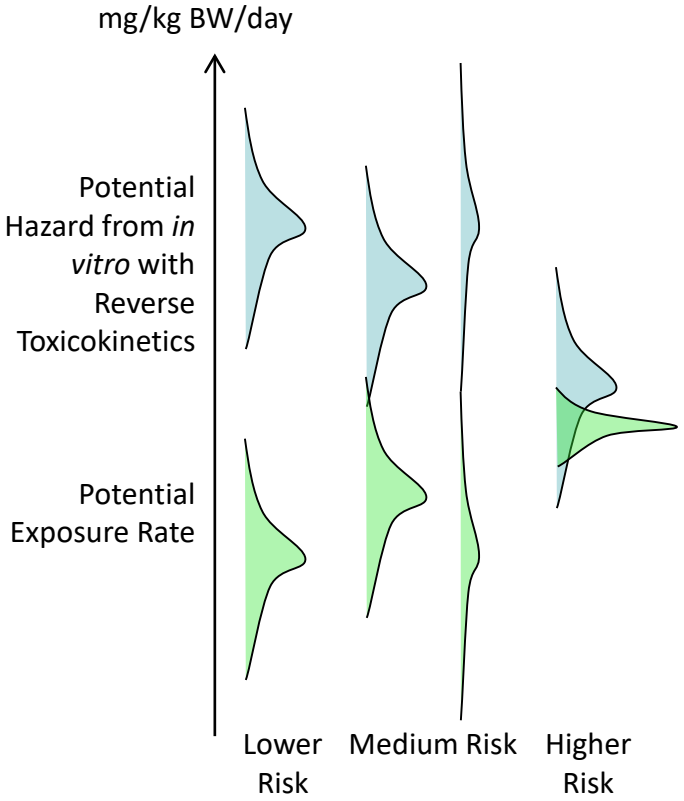
"Translation of high-throughput data into risk-based rankings is an important application of exposure data for chemical priority-setting. Recent advances in high-throughput toxicity assessment, notably the ToxCast and Tox21 programs… and in high-throughput computational exposure assessment… have enabled first-tier risk-based rankings of chemicals on the basis of margins of exposure…"



High-Throughput Risk Prioritization

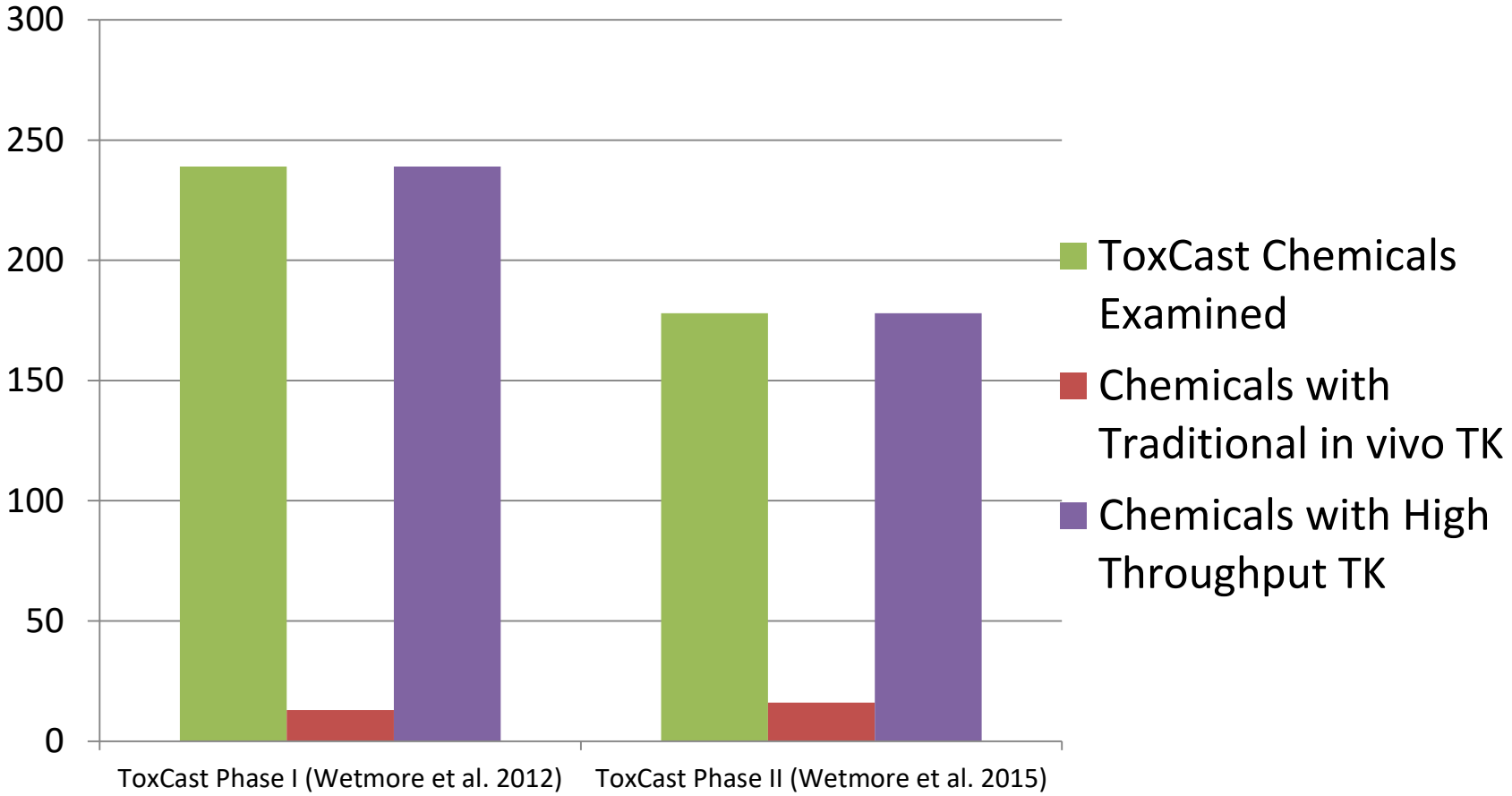
- High throughput risk prioritization based upon *in vitro-in vivo* extrapolation (IVIVE) requires (e.g., NRC, 1983):





The Need for *In Vitro* Toxicokinetics

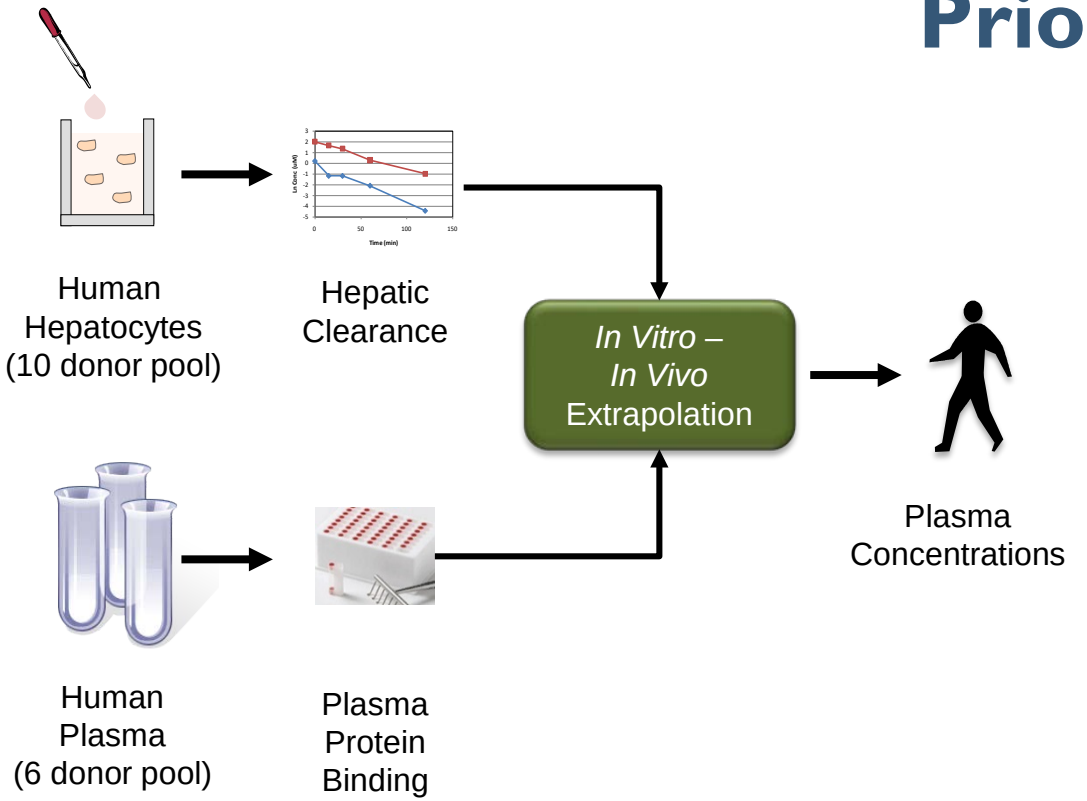
Most chemicals do not have TK data – Wetmore et al. (2012…) use *in vitro* methods adapted from pharma to fill gaps

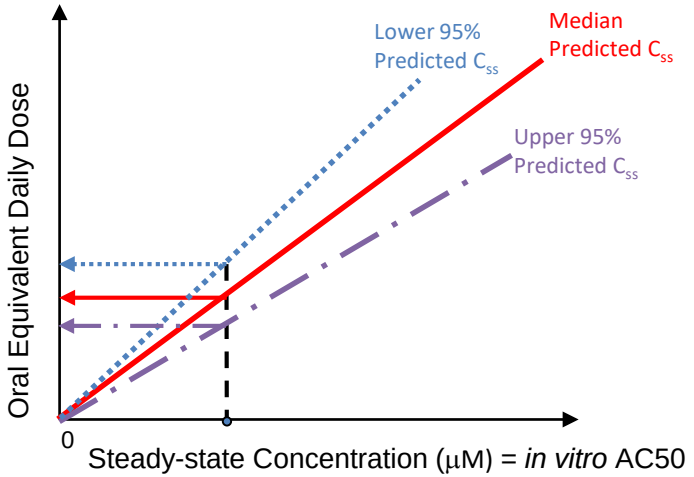


High Throughput Toxicokinetics (HTTK)

- In order to address greater numbers of chemicals we collect *in vitro*, high throughput toxicokinetic (HTTK) data (Rotroff et al., 2010, Wetmore et al., 2012, 2015)
- HTTK methods have been used by the pharmaceutical industry to determine range of efficacious doses and to prospectively evaluate success of planned clinical trials (Jamei, *et al.*, 2009; Wang, 2010)
- The **primary goal** of HTTK is to provide a human dose context for bioactive *in vitro* concentrations from HTS (*i.e., in vitro-in vivo* extrapolation, or **IVIVE**) (e.g., Wetmore et al., 2015)
- **Secondary goal** is to provide **open source data and models** for evaluation and use by the broader scientific community (Pearce et al, 2017a)

In Vitro Toxicokinetics for Prioritization





Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (in prep.) + ~300 chemicals

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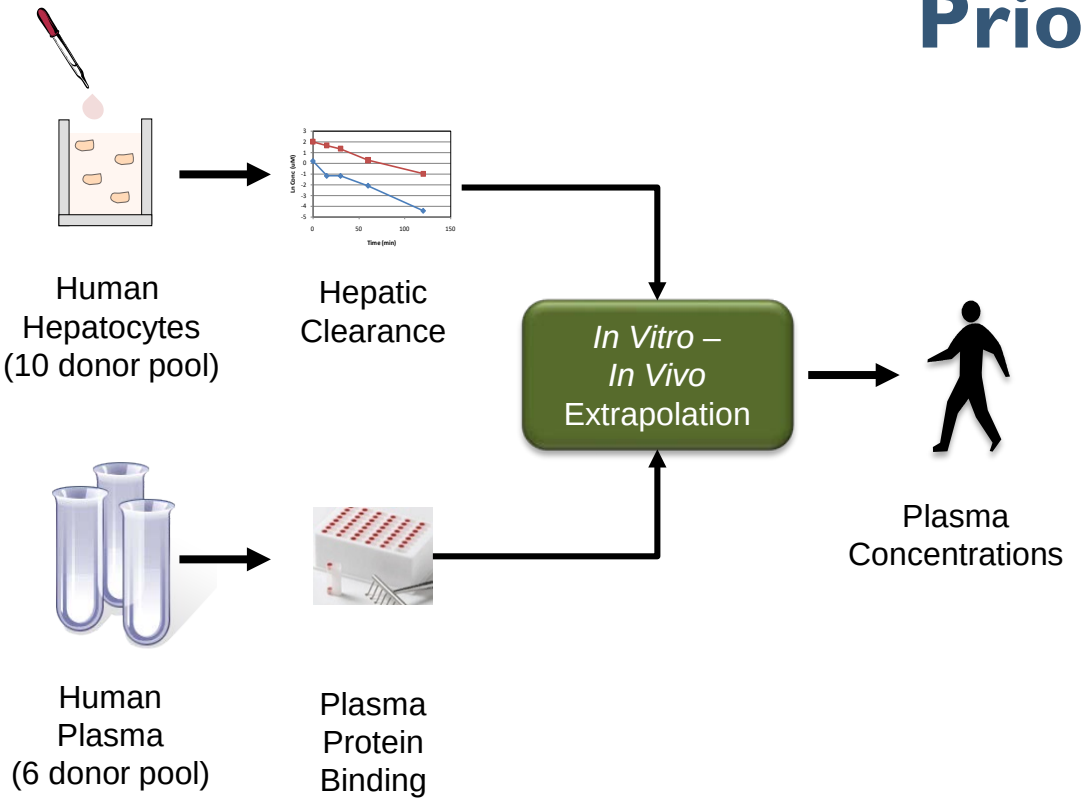

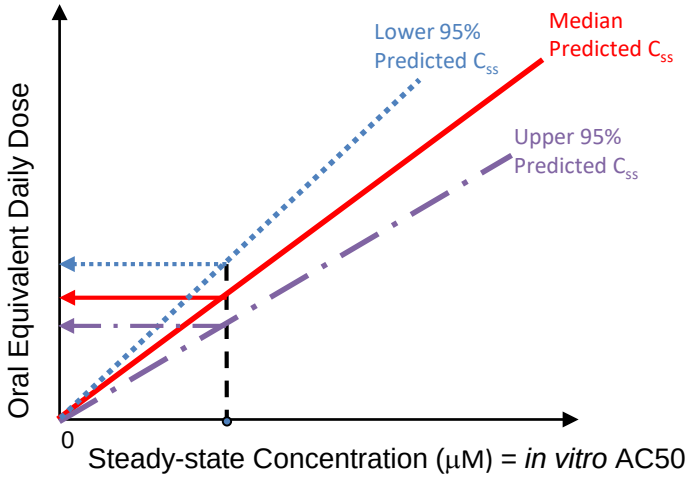

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httk: A Public, Open Source Tool

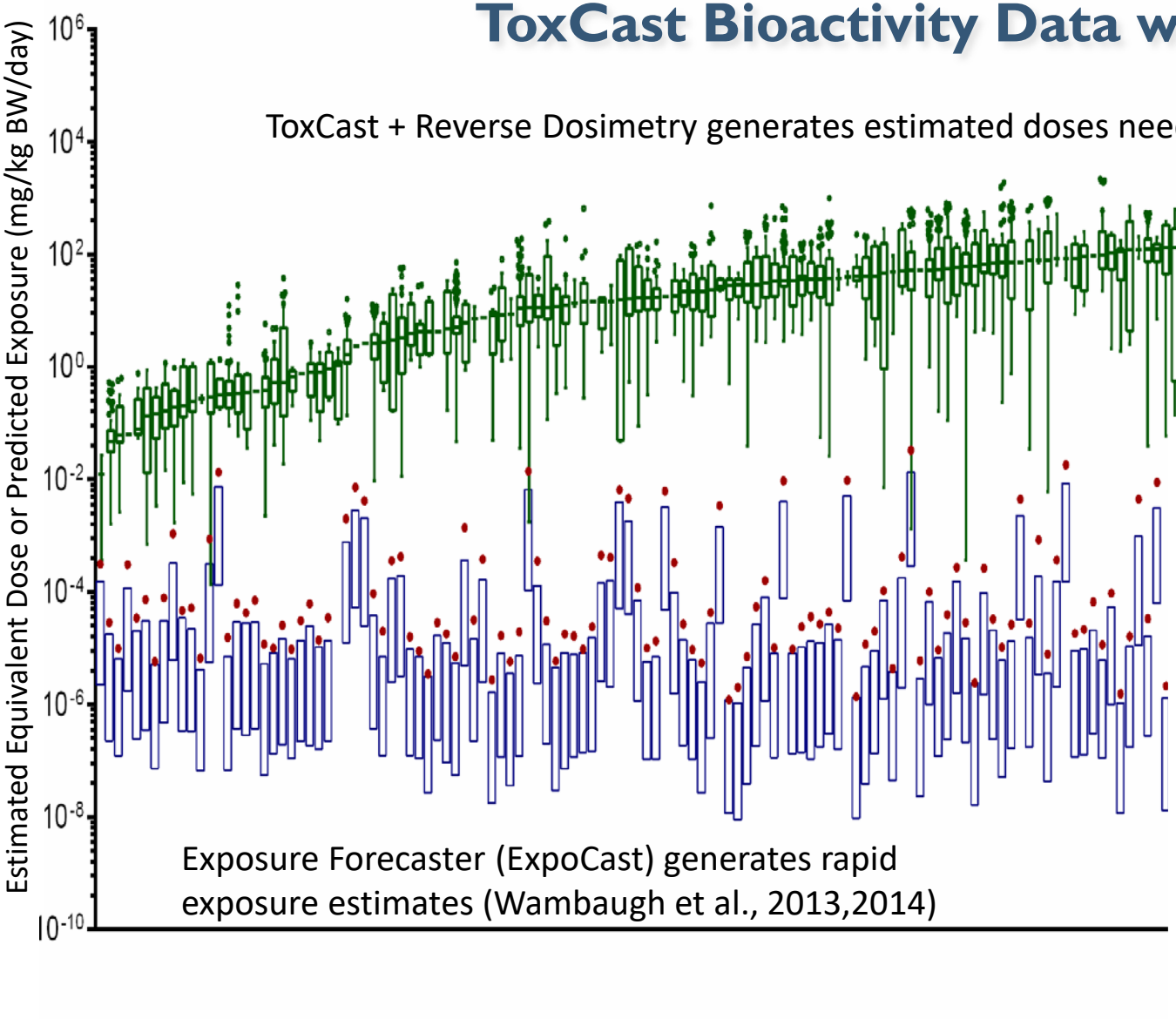
`httk: High-Throughput Toxicokinetics`

Functions and data tables for simulation and statistical analysis of chemical toxicokinetics ("TK") using data obtained from relatively high throughput, in vitro studies. Both physiologically-based ("PBTK") and empirical (e.g., one compartment) "TK" models can be parameterized for several hundred chemicals and multiple species. These models are solved efficiently, often using compiled (C-based) code. A Monte Carlo sampler is included for simulating biological variability and measurement limitations. Functions are also provided for exporting "PBTK" models to "SBML" and "JARNAC" for use with other simulation software. These functions and data provide a set of tools for in vitro-in vivo extrapolation ("IVIVE") of high throughput screening data (e.g., ToxCast) to real-world exposures via reverse dosimetry (also known as "RTK").

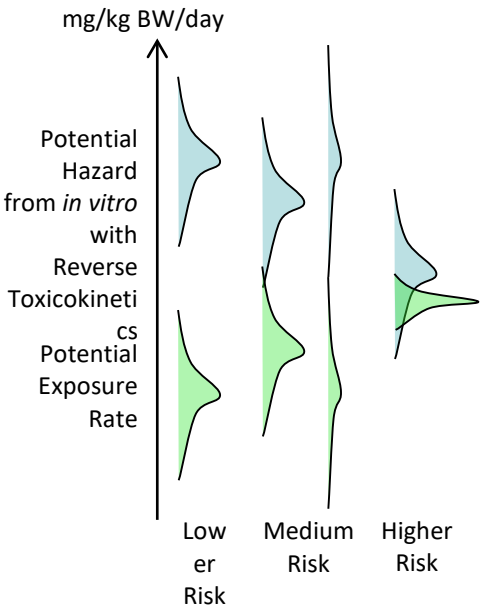
https://CRAN.R-project.org/package=httk

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with Exposure

ToxCast + Reverse Dosimetry generates estimated doses needed to cause bioactivity



Exposure Forecaster (ExpoCast) generates rapid exposure estimates (Wambaugh et al., 2013,2014)



Wetmore *et al.*, Tox. Sci, 2015

High Throughput Toxicokinetics (HTTK) for Statistical Analysis

Download R:

https://www.r-project.org/

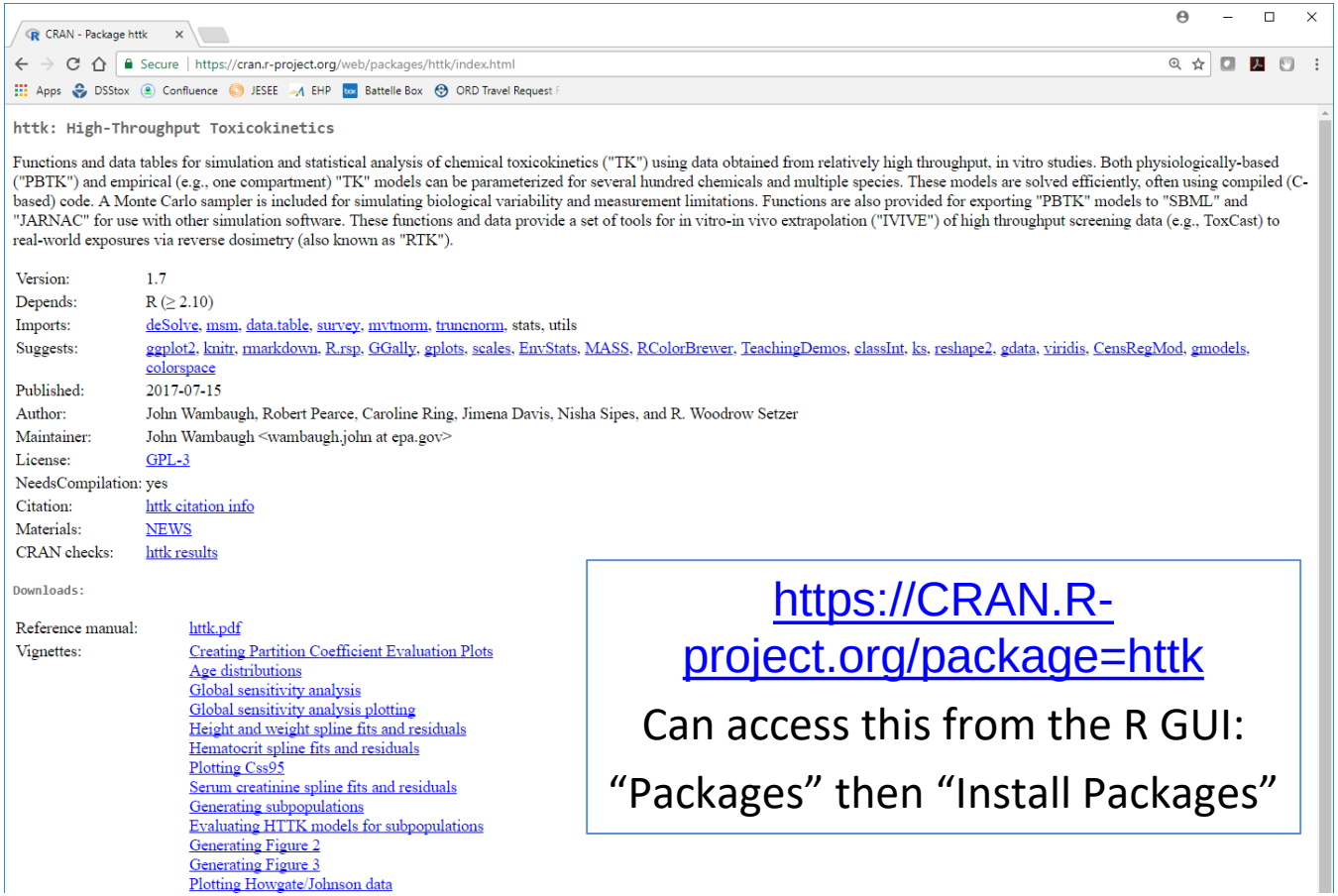
within R, type:

```
install.packages("httk")
```

Then

```
library("httk")
```

- "httk" R Package for IVIVE and **PBTK**
- 553 chemicals to date
- 100's of additional chemicals being studied
- Pearce *et al.* (2017a) provides documentation and examples
- Built-in vignettes provide further examples of how to use many functions



https://CRAN.R-project.org/package=httk

Can access this from the R GUI:

"Packages" then "Install Packages"

Why Build Another Generic PBTK Tool?

In addition to new Population Lifecourse Exposure-To-Health-Effects Model Suite, various groups have been generating generic PBPTK models for some time:

	SimCYP	ADMET Predictor / GastroPlus	MEGen	IndusChemFate	httk
Maker	SimCYP Consortium / Certara	Simulations Plus	UK Health and Safety Laboratory	Cefic LRI	US EPA
Availability	License, but inexpensive for research	License, but inexpensive for research	Free: http://xnet.hsl.gov.uk/megen	Free: http://cefic-lri.org/lri_toolbox/induschemfate/	Free: https://CRAN.R-project.org/package=httk
Open Source	No	No	**Yes**	No	**Yes**
Default PBPK Structure	**Yes**	**Yes**	No	**Yes**	**Yes**
Expandable PBPK Structure	No	No	**Yes**	No	No
Population Variability	**Yes**	No	No	No	**Yes**
Batch Mode	**Yes**	**Yes**	No	No	**Yes**
Graphical User Interface	**Yes**	**Yes**	**Yes**	Excel	No
Physiological Data	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Chemical-Specific Data Library	Many Clinical Drugs	No	No	15 Environmental Compounds	543 Pharmaceutical and ToxCast Compounds
Ionizable Compounds	**Yes**	**Yes**	Potentially	No	**Yes**
Export Function	No	No	Matlab and AcslX	No	SBML and Jarnac
R Integration	No	No	No	No	**Yes**
Easy Reverse Dosimetry	**Yes**	**Yes**	No	No	**Yes**
Future Proof XML	No	No	**Yes**	No	No

We want to do a statistical analysis (using R) for as many chemicals as possible

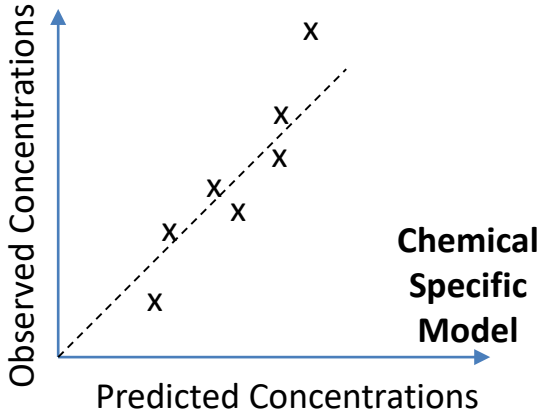
Doing Statistical Analysis with HTTK

- If we are to use HTTK, we need confidence in predictive ability
- In drug development, HTTK methods estimate therapeutic doses for clinical studies – predicted concentrations are typically on the order of values measured in clinical trials (Wang, 2010)
  - For most compounds in the environment there will be no clinical trials
- Uncertainty must be well characterized
  - We compare to *in vivo* data to get **empirical estimates of HTTK uncertainty**
  - ORD has both compiled existing (literature) TK data (Wambaugh *et al*., 2015) and conducted new experiments in rats on chemicals with HTTK *in vitro* data (Wambaugh *et al*., submitted)
  - Any approximations, omissions, or mistakes should work to increase the estimated uncertainty when evaluated systematically across chemicals

Model Evaluation

- In order to evaluate a **chemical-specific TK model** for "chemical x" you can compare the predictions to *in vivo* measured data
  - Can estimate bias
  - Can estimate uncertainty
  - Can consider using model to extrapolate to other situations (dose, route, physiology) where you don't have data

- However, we do not typically have TK data



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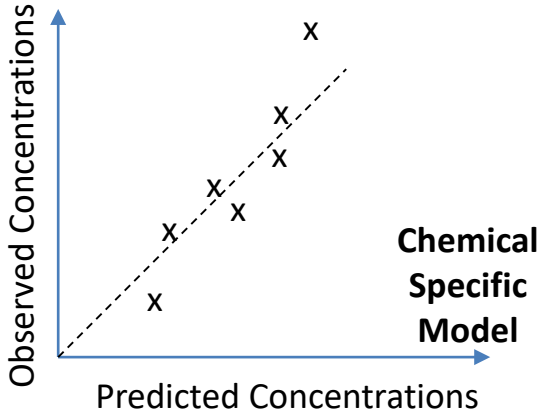
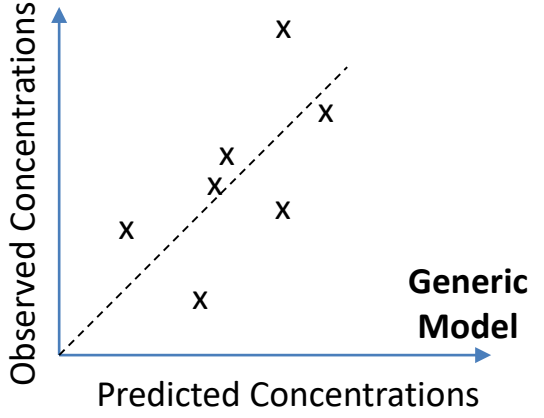
- We can parameterize a **generic TK model**, and evaluate that model for as many chemicals as we do have data
  - We do expect larger uncertainty, but also greater confidence in model implementation
  - Estimate bias and uncertainty, and try to correlate with chemical-specific properties
  - Can again consider using model to extrapolate to other situations (chemicals without *in vivo* data)





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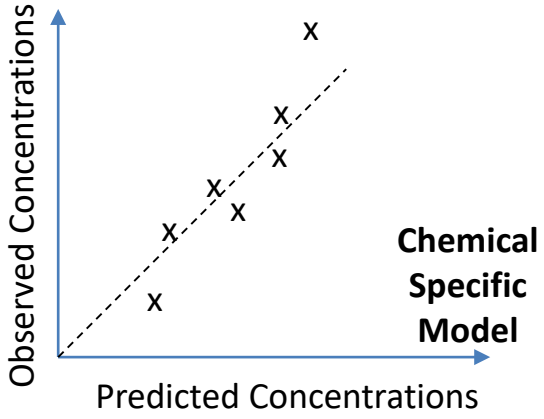

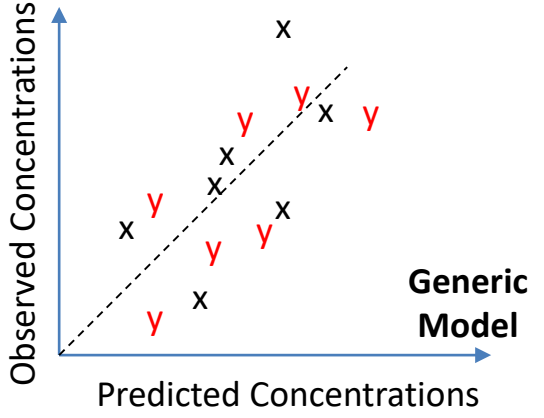

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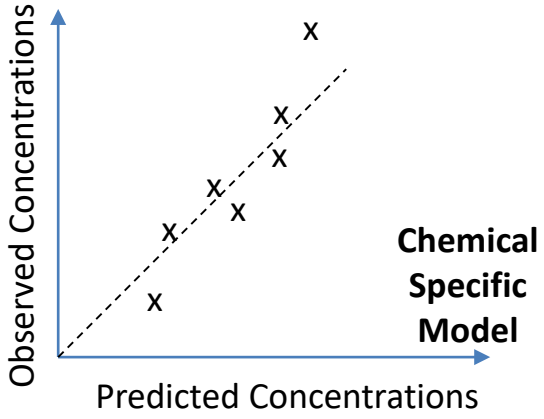
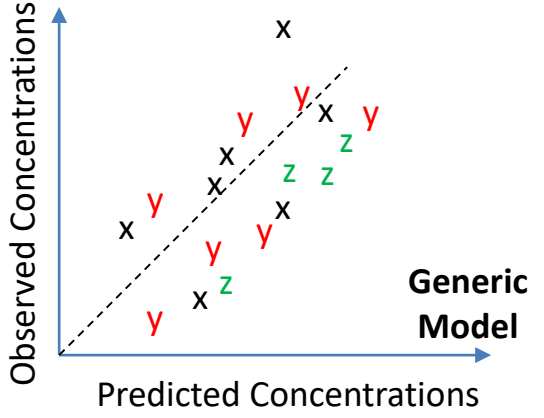
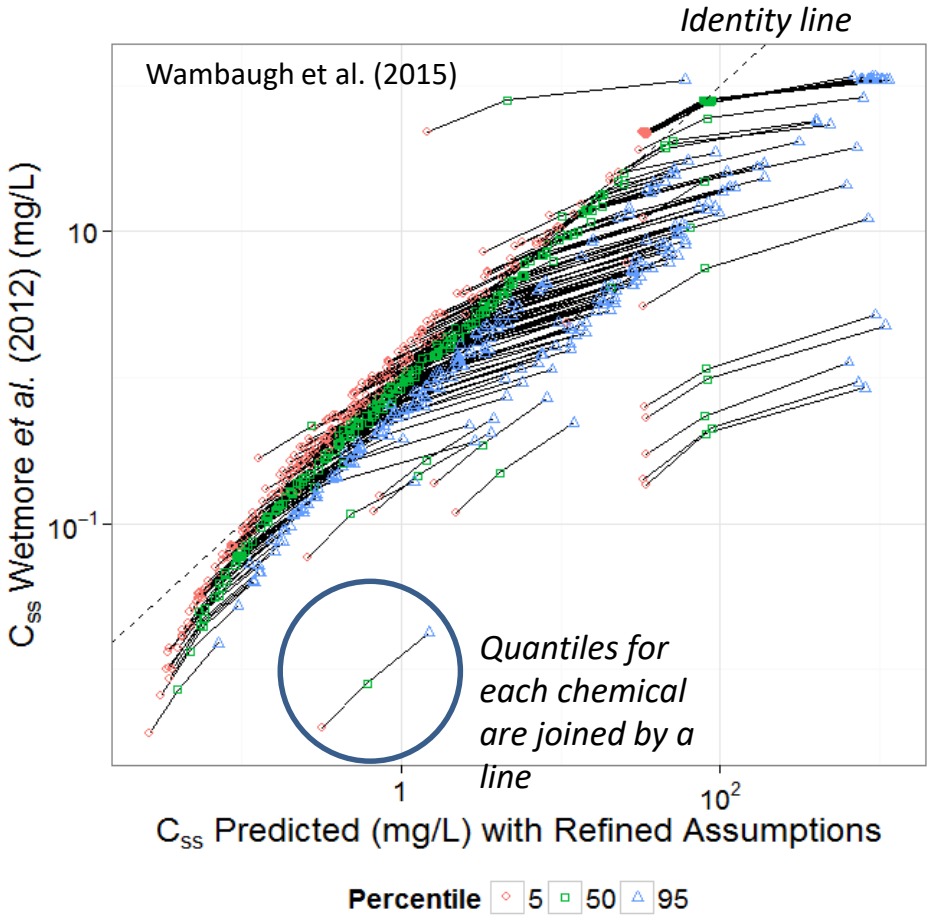


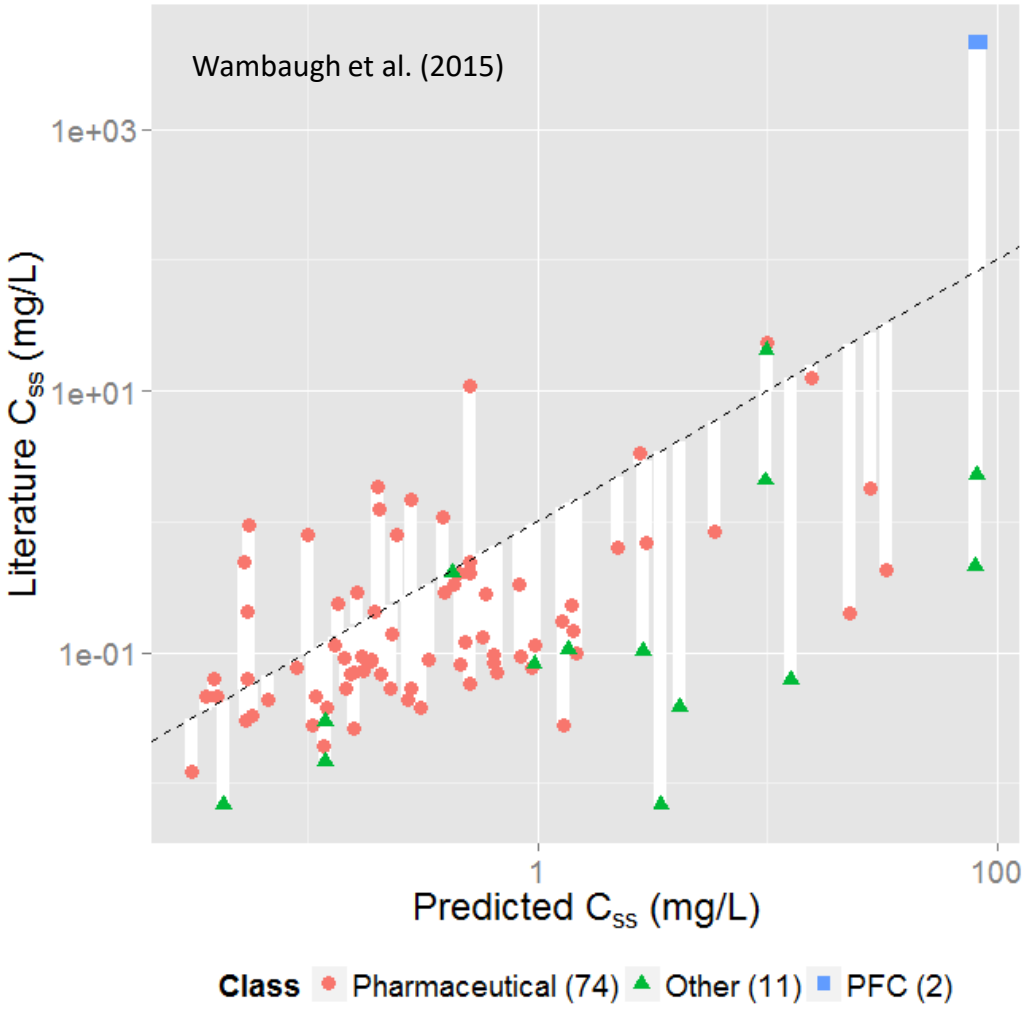


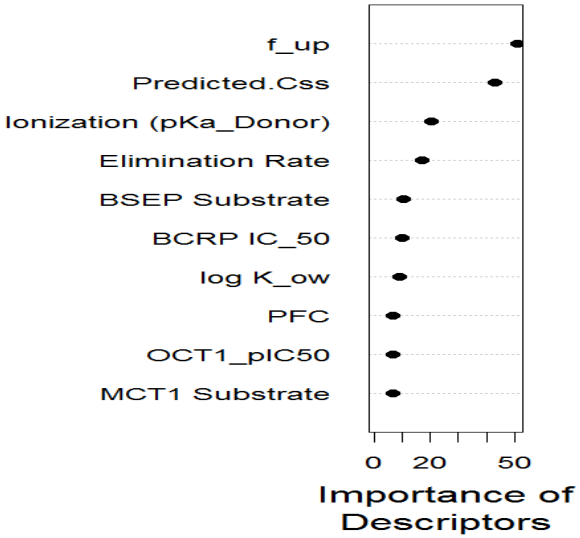
Comparison Between httk and SimCYP

- In the Rotroff *et al*. (2010) and Wetmore et al. (2012,2013,2014,2015) papers SimCYP was used to predict distributions of C_{ss} from *in vitro* data
    - We show that "httk" can reproduce the results from those publications for most chemicals using our implementation of Monte Carlo.
- Any one chemical's median, 5th and 95th quantiles are connected by a line.
- The RED assay for measuring protein binding fails in some cases because the amount of free chemical is below the limit of detection
    - A default value of 0.5% free was used
    - Now we use random draws from a uniform distribution from 0 to 1%.



Using *in vivo* Data to Evaluate RTK

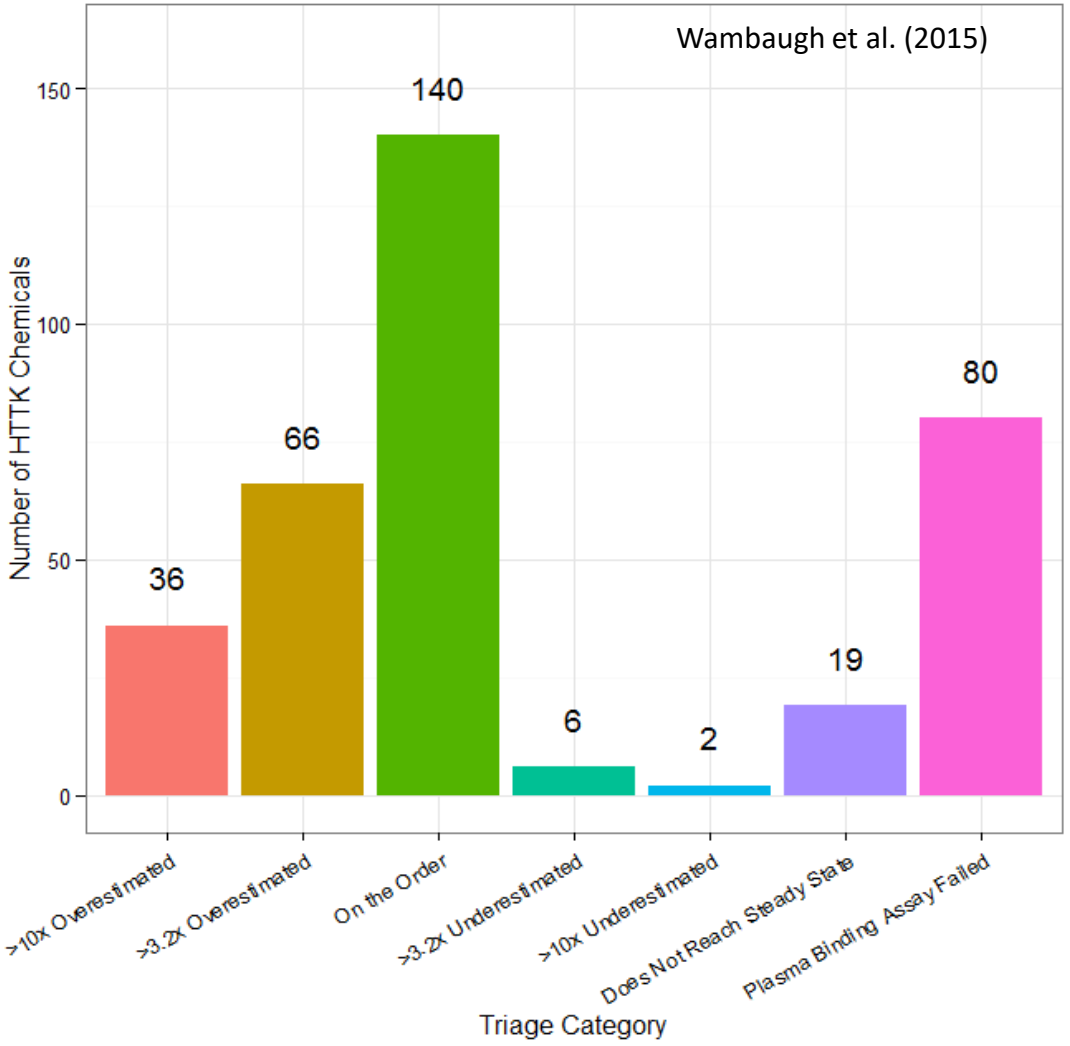




- When we compare the C_{ss} predicted from *in vitro* HTTK with *in vivo* C_{ss} values determined from the literature we find limited correlation (R^2 ~0.34)
- The dashed line indicates the identity (perfect predictor) line:
  - Over-predict for 65
  - Under-predict for 22
- The white lines indicate the discrepancy between measured and predicted values (the residual)

Toxicokinetic Triage

- Through comparison to *in vivo* data, a cross-validated (random forest) predictor of success or failure of HTTK has been constructed
- Add categories for chemicals that do not reach steady-state or for which plasma binding assay fails
- All chemicals can be placed into one of seven confidence categories
- Plurality of chemicals end up in the “on the order” bin (within a factor of 3.2x) which is consistent with Wang (2010)



Installing "httk"

```
install.packages("httk")

library(httk)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for
Acetochlor (published value):
calc_mc_css(chem.cas="34256-82-1")

#State-state oral equivalent dose (mg/kg BW/day) to produce 0.1 uM serum concentration for
human, 0.95 quantile, for Acetochlor (calculated value):
calc_mc_oral_equiv(0.1,chem.cas="34256-82-1")

# Should produce error:
calc_mc_css(chem.name="34256-82-1")

#Capitalization shouldn't matter:
calc_mc_css(chem.name="acetochlor")
calc_mc_css(chem.name="Acetochlor")

# What's going on?
help(calc_mc_css)
```

Interspecies Extrapolation Examples

```
#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for human for Acetochlor
(calculated value):
calc_mc_css(chem.cas="34256-82-1")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor (should
produce errors since there is no published value, 0.5 quantile only):
get_wetmore_css(chem.cas="34256-82-1",species="Rat")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for rat for Acetochlor
(calculated value):
calc_mc_css(chem.cas="34256-82-1",species="Rat")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor
(published value):
get_wetmore_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.5 quantile for rat for Acetochlor
(calculated value):
calc_mc_css(chem.cas="34256-82-1",species="Rat",which.quantile=0.5)

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for mouse for Acetochlor
(should produce error since there is no published value, human and rat only):
get_wetmore_css(chem.cas="34256-82-1",species="Mouse")

#Steady-state concentration (uM) for 1 mg/kg/day for 0.95 quantile for  mouse for Acetochlor
(calculated value):
calc_mc_css(chem.cas="34256-82-1",species ="Mouse")
calc_mc_css(chem.cas="34256-82-1",species ="Mouse",default.to.human=T)
```

Help Files

Every function has a help file

```
help(add_chemtable)
```

Add a table of chemical information for use in making httk predictions.

Description

This function adds chemical-specific information to the table chem.physical_and_invitro.data. This table is queried by the model parameterization functions when attempting to parameterize a model, so adding sufficient data to this table allows additional chemicals to be modeled.

Usage

```
add_chemtable(new.table, data.list, current.table=NULL, reference=NULL,species=NULL,
overwrite=F)
```

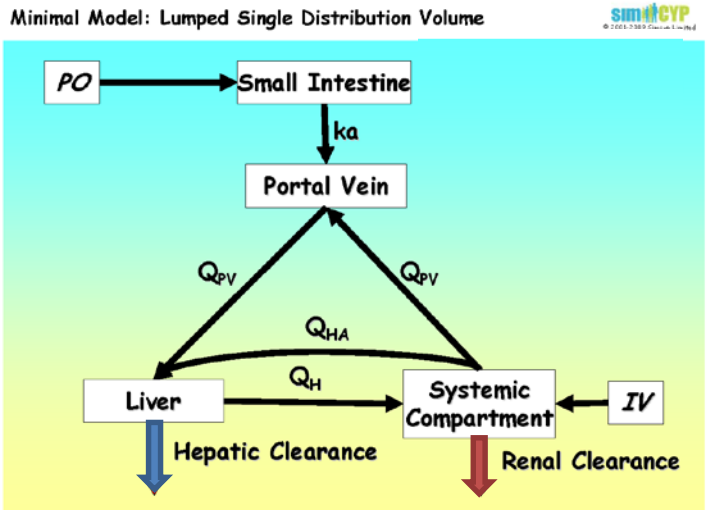
Arguments

new.table	Object of class data.frame containing one row per chemical, with each chemical minimally by described by a CAS number.
data.list	This list identifies which properties are to be read from the table. Each item in the list should point to a column in the table new.table. Valid names in the list are: 'Compound', 'CAS', 'DSSTox.GSID' 'SMILES.desalt', 'Reference', 'Species', 'MW', 'logP', 'pKa_Donor', 'pKa_Accept', 'logMA', 'Clint', 'Clint.pValue', 'Funbound.plasma', 'Fgutabs', 'Rblood2plasma'. Note that Rblood2plasma (Ratio blood to plasma) is currently not used.

Pearce et al. (2017a)

Variability in this Steady-State TK Model

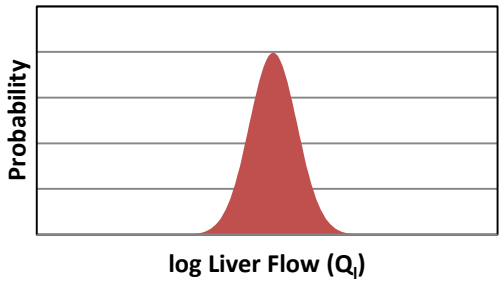
Jamei *et al*. (2009)

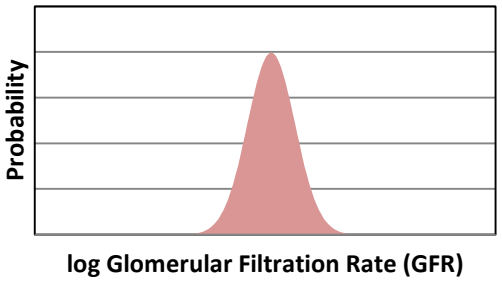


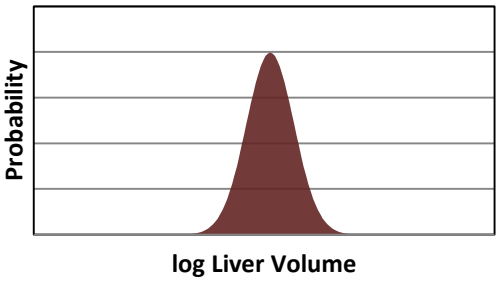
$$C_{ss} = \frac{\text{oral dose rate}}{\underbrace{(GFR * F_{ub})}_{\text{(Passive) Renal Clearance}} + \underbrace{\left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}_{\text{Hepatic Clearance (Metabolism)}}}$$

- *In vitro* clearance (µL/min/10^6 hepatocytes) is scaled to a whole organ clearance using the density of hepatocytes per gram of liver and the volume of the liver (which varies between individuals)
- Glomerular filtration rate (GFR) and blood flow to the liver (Q_l) both vary from individual to individual
- Further assume that measured HTTK parameters have 30% coefficient of variation

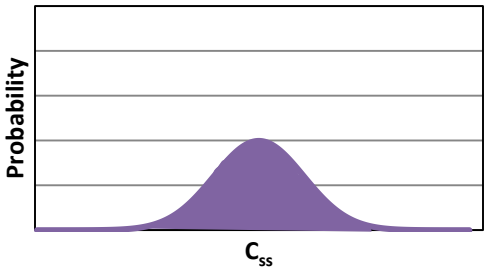
Monte Carlo (MC) Approach to Variability

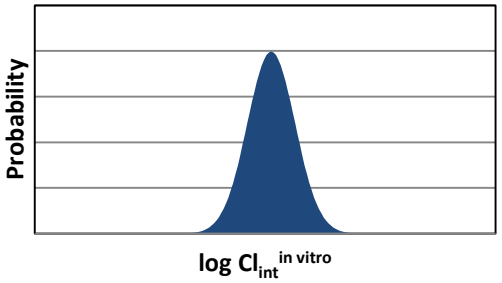


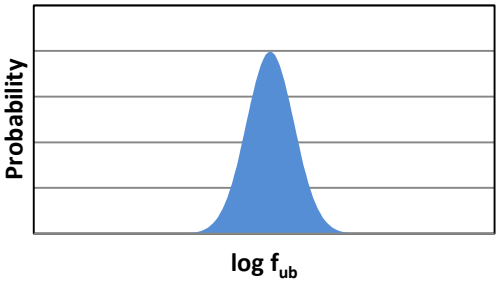




$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

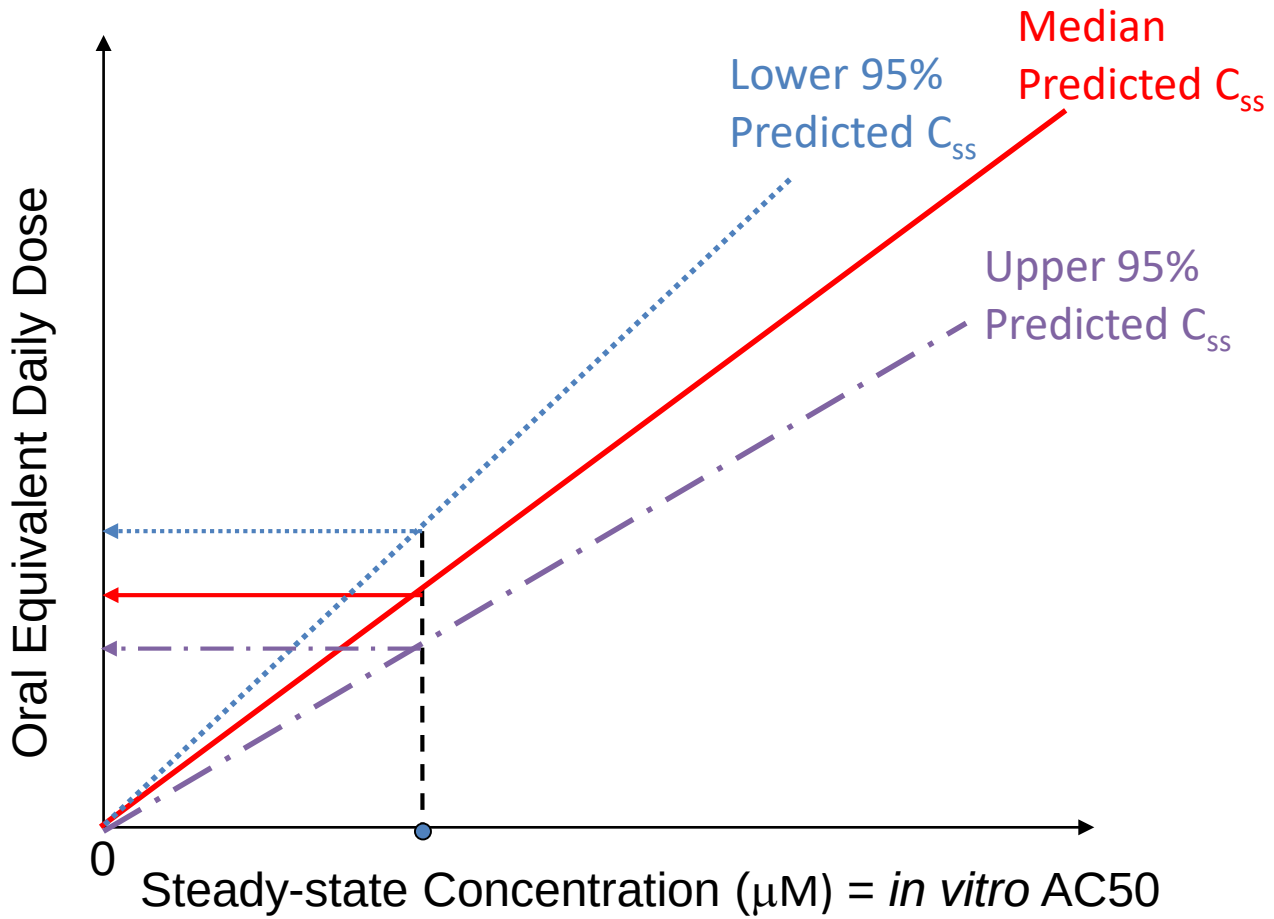






Wetmore *et al.* (2012)

Steady-State *In Vitro-In Vivo* Extrapolation (IVIVE)



- The higher the predicted C_{ss}, the lower the oral equivalent dose, so the upper 95% predicted C_{ss} from the MC has a lower oral equivalent dose

McNally et al. (2014) Linear Regressions for Population Simulation

Toxicology 315 (2014) 70–85

Contents lists available at ScienceDirect

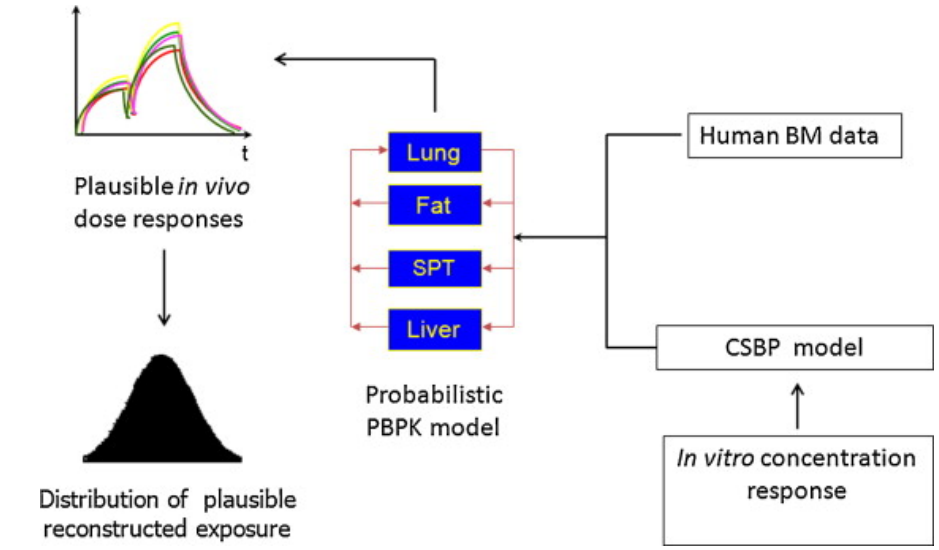
Toxicology

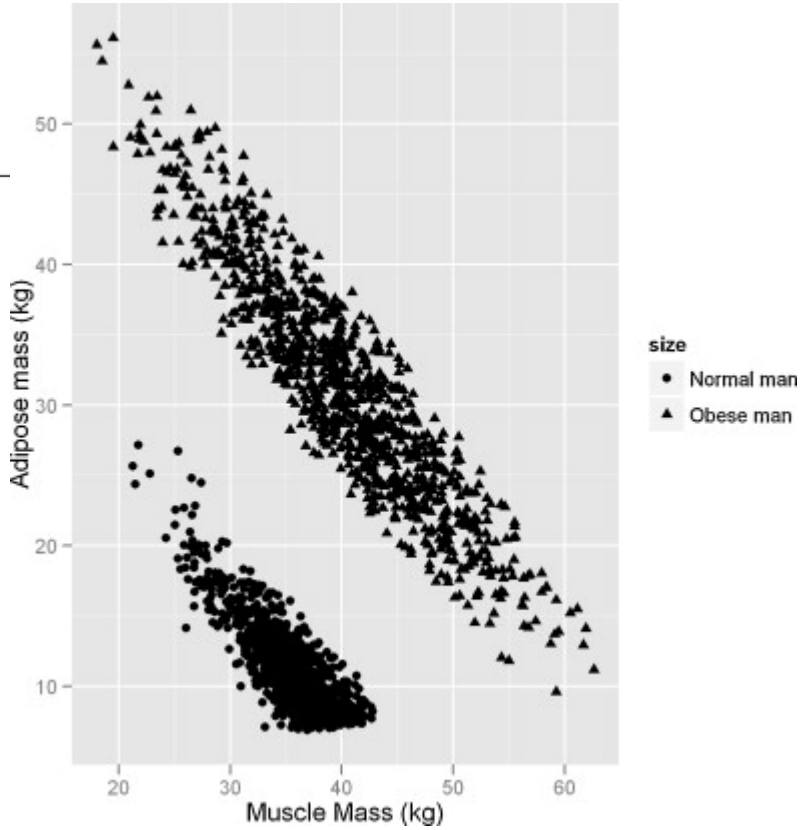
journal homepage: www.elsevier.com/locate/toxicol

PopGen: A virtual human population generator

Kevin McNally[a], Richard Cotton[b], Alex Hogg[a], George Loizou[a,*]

[a] Health & Safety Laboratory, Buxton, Derbyshire, UK
[b] TDL Ltd, Buxton, Derbyshire, UK





Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Ring *et al*. (2017)

Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Use equations from literature
(McNally *et al.*, 2014)
(+ residual marginal variability)

Ring *et al*. (2017)

Modern U.S. Population Simulator for HTTK

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

Sex
Race/ethnicity
Age
Height
Weight
Serum creatinine

Use equations from literature
(McNally *et al.*, 2014)
(+ residual marginal variability)

Predict physiological quantities

Tissue masses
Tissue blood flows
GFR (kidney function)
Hepatocellularity

Ring *et al*. (2017)

Generating demographic subgroups

User can specify….	Default if not specified
Age limits	0-79 years
Sex (# males, # females)	NHANES proportions
Race/ethnicity (5 NHANES categories)	NHANES proportions
BMI/weight categories	NHANES proportions

- NHANES quantities sampled from appropriate *conditional* distribution (given specifications)
  - Physiological parameters predicted accordingly

Ring *et al*. (2017)

NHANES Demographic Examples

```
library(httk)

# Oral equivalent (mg/kg/day) for in vitro activity of 1 µM for Acetochlor
calc_mc_oral_equiv(1,chem.cas="34256-82-1")

# Oral equivalent (mg/kg/day) for NHANES “Mexican American” Population
calc_mc_oral_equiv(1,chem.cas="34256-82-1", reths = "Mexican American")

# Oral equivalent (mg/kg/day) for NHANES “Mexican American” Population aged 18-25 years
calc_mc_oral_equiv(1,chem.cas="34256-82-1",agelim_years=c(18,25),reths = "Mexican
American")

# Probably too few individuals in NHANES for direct resampling (“dr”) so use virtual
individuals (“vi”) resampling method:
calc_mc_oral_equiv(1,chem.cas="34256-82-1",method="vi",agelim_years=c(18,25),reths =
"Mexican American")
```

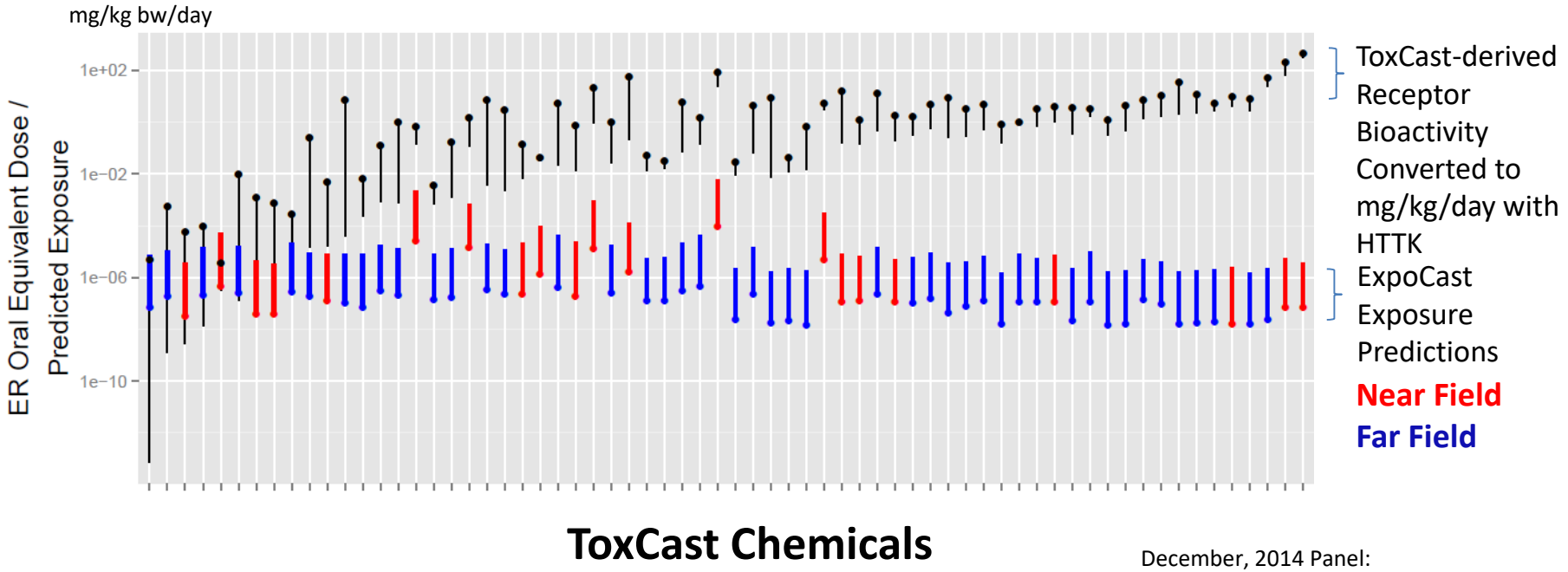
Can also specify gender, weight categories, and kidney function

Ring *et al*. (2017)

United States Environmental Protection Agency

High Throughput Risk Prioritization for the Total Population

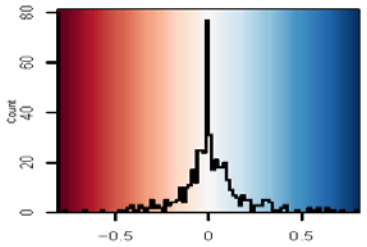
High throughput toxicokinetics bridges high throughput screening and exposure estimates



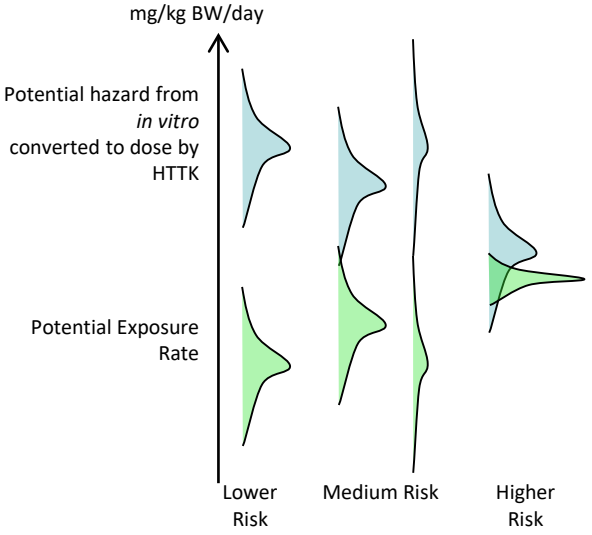
December, 2014 Panel:
"Scientific Issues Associated with Integrated Endocrine Bioactivity and Exposure-Based Prioritization and Screening"

Life-stage and Demographic Specific Predictions

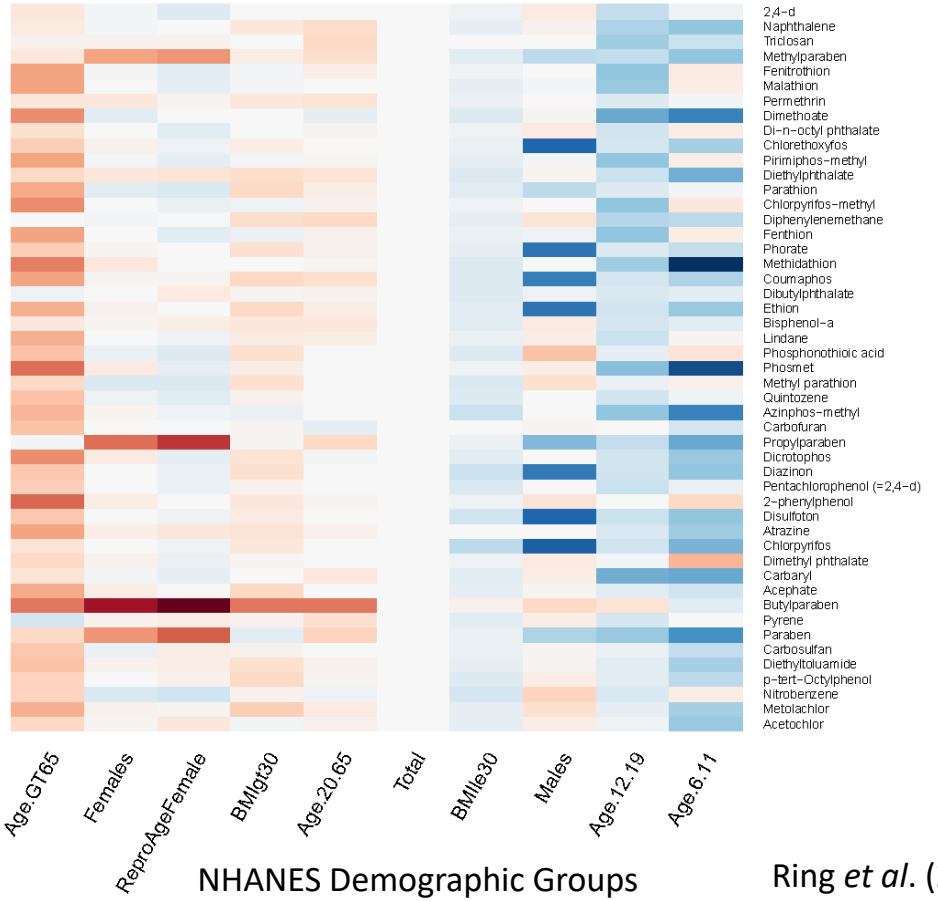
- We use HTTK to calculate margin between bioactivity and exposure for specific populations
- Most NHANES chemicals do not have traditional PK models (Strope et al., 2018)



Change in Risk



Change in Activity : Exposure Ratio



NHANES Chemicals

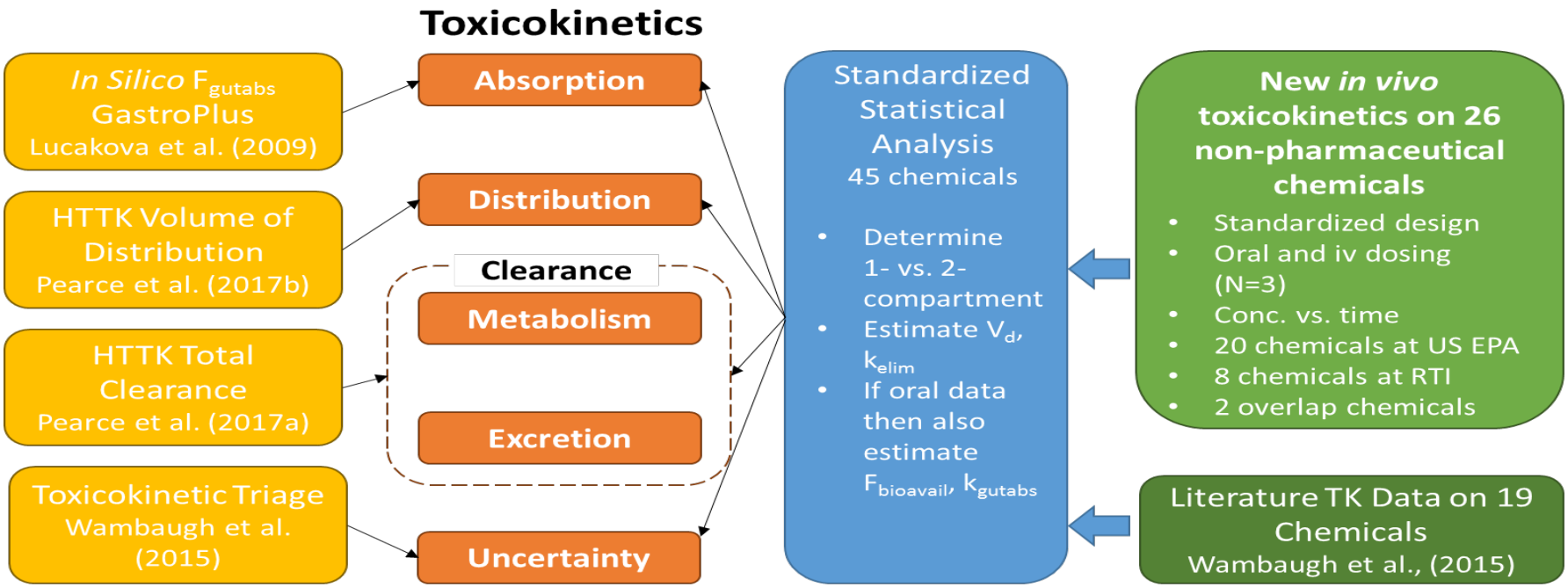
NHANES Demographic Groups

Ring *et al*. (2017)

Evaluating HTTK Predictions

We collected new *in vivo* data for 26 chemicals more commonly associated with non-therapeutic and/or unintentional exposure

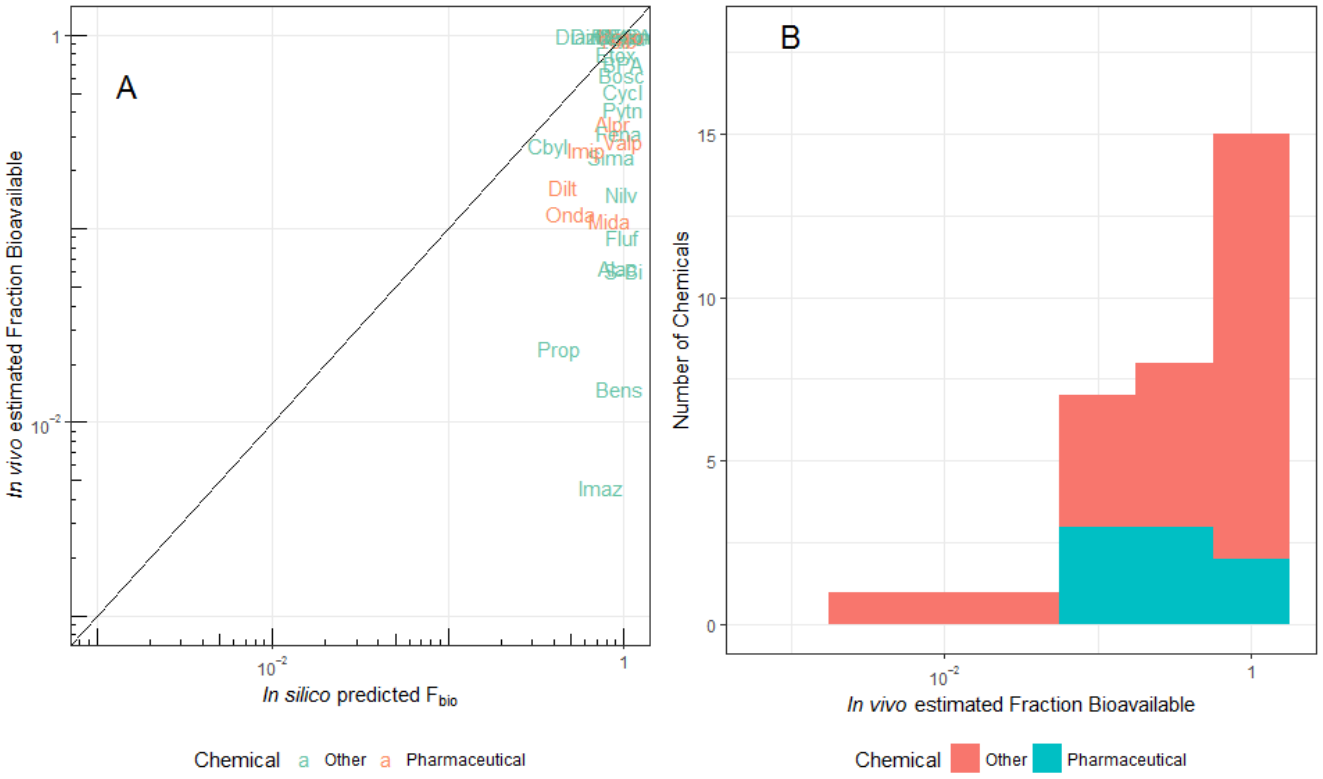
Minimal design – six animals per study (3 dosed per oral / 3 iv)



In Vivo Work led by Mike Hughes (EPA/NHEERL) and Tim Fennell (RTI)

Wambaugh et al., submitted

Evaluating *In Silico* Oral Bioavailability Predictions with *In Vivo* Data



- *In silico* methods developed for pharmaceuticals do not seem to do a good job of predicting oral bioavailability for environmental chemicals
- Predictions were made without the benefit of in vitro assays that can inform absorption (i.e., CACO-2 membrane permeability)
- CACO-2 permeability is now being measured for HTTK chemicals (Derek Angus, Cyprotex)

Bioavailability predictions from GastroPlus (Nisha Sipes)

Wambaugh et al., submitted

Does My Chemical Have HTTK Data?

All data on chemicals A, B, C

```
subset(get_cheminfo(info="all"),Compound%in%c("A","B","C"))
```

```
> library(httk)
> get_cheminfo()
  [1] "2971-36-0"   "94-75-7"     "94-82-6"     "90-43-7"     "1007-28-9"
  [6] "71751-41-2"  "30560-19-1"  "135410-20-7" "34256-82-1"  "50594-66-6"
 [11] "15972-60-8"  "116-06-3"    "834-12-8"    "33089-61-1"  "101-05-3"
 [16] "1912-24-9"   "86-50-0"     "131860-33-8" "22781-23-3"  "1861-40-1" …
> get_cheminfo(info="all")
```

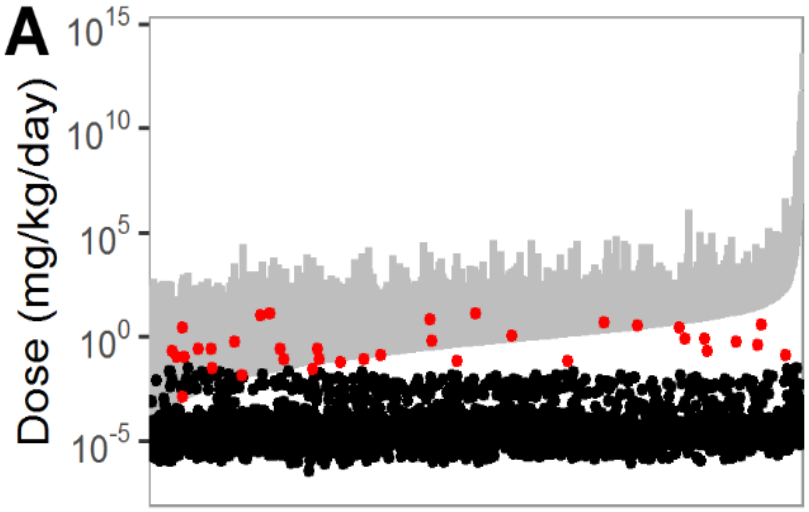
Compound	CAS	logP	pKa_Accept	pKa_Donor	MW	Human.Clint	Human.Clint.pValue	Human.Funbound.plasma	DSSTox_Substance_Id	Structure_Formula	Substance_Type
2,4-d	94-75-7	2.81	<NA>	2.81	221.03	0	0.149	0.04	DTXSID0020442	C8H6Cl2O3	Single Compound
2,4-db	94-82-6	3.53	<NA>	4.5	249.09	0	0.104	0.01	DTXSID7024035	C10H10Cl2O3	Single Compound
2-phenylphenol	90-43-7	3.09	<NA>	10.6	170.211	2.08	0.164	0.04	DTXSID2021151	C12H10O	Single Compound
6-desisopropylatrazine	1007-28-9	1.15	1.59	<NA>	173.6	0	0.539	0.46	DTXSID0037495	C5H8ClN5	Single Compound

Is a chemical available?

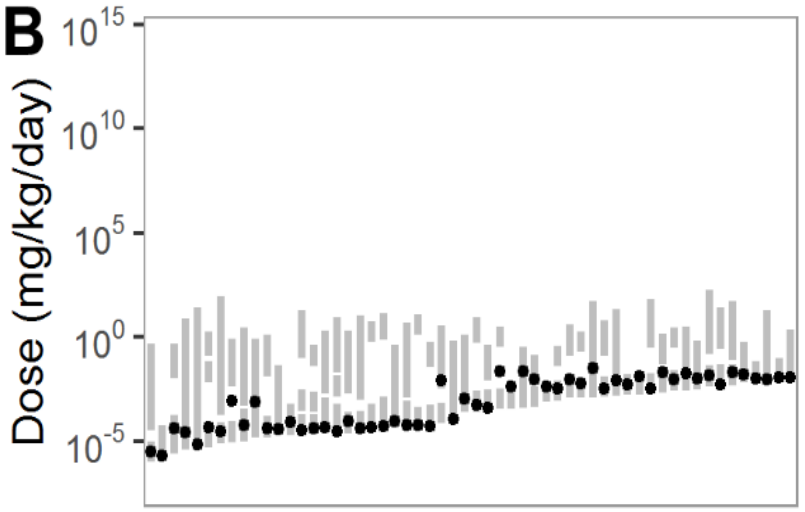
```
> "80-05-7" %in% get_cheminfo()
[1] TRUE
```

In Silico HTTK Predictions

- Tox21 has screened >8000 chemicals – Sipes *et al.* (2017) wanted to compare *in vitro* active concentrations with HTTK predicted maximum plasma concentrations with high throughput exposure predictions from Wambaugh et al. (2014)
  - "httk" package only has ~500 chemicals
- Used Simulations Plus ADMet Predictor to predict for entire library (supplemental table) and used add_chemtable() function to add into "httk" package
- Data available as on-line with new toolbox: https://sandbox.ntp.niehs.nih.gov/ivive/



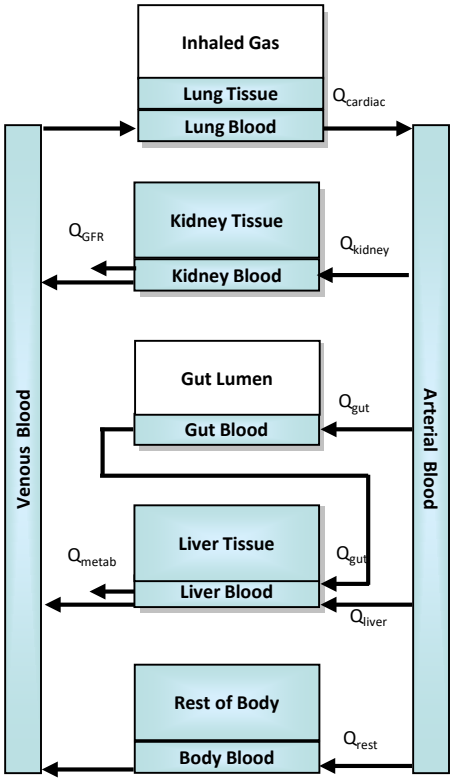
Dose range for all 3925 Tox21 compounds eliciting a 'possible'-to-'likely' human *in vivo* interaction alongside estimated daily exposure



56 compounds with potential *in vivo* biological interaction at or above estimated environmental exposures

Figure from Sipes *et al.*, (2017)

A General Physiologically-based Toxicokinetic (PBTK) Model

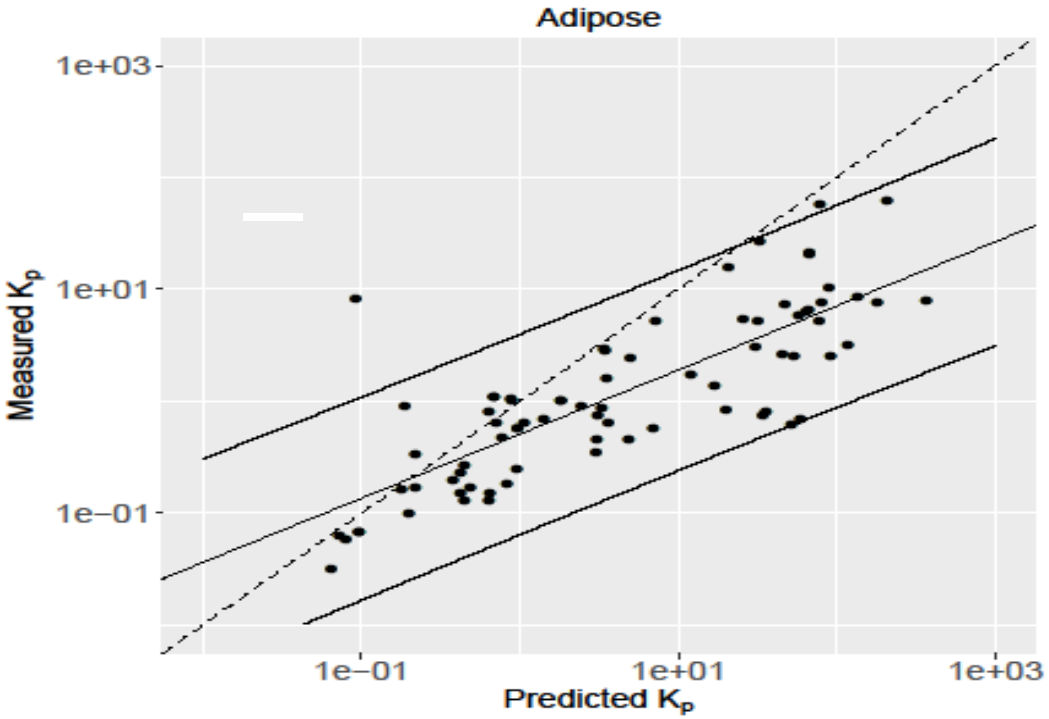


- "httk" also includes a generic PBTK model
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.
- Blood flows move the chemical throughout the body. The total blood flow to all tissues equals the cardiac output.
- The only ways chemicals "leave" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

Basic PK Statistics Examples

```
library(httk)
#A Function to get PK summary statistics from the PBPK model:
help(calc_stats)
# 28 day human study (20 mg/kg/day) for Bisphenol A:
calc_stats(days=28,chem.name="bisphenol a", dose=20)
     Human plasma concentrations returned in uM units.
     AUC is area under plasma concentration curve in uM * days units with Rblood2plasma =
     0.79 .
     $AUC
     [1] 44.82138
     $peak
     [1] 23.16455
     $mean
     [1] 1.600764
# Units default to μM but can use mg/L:
calc_stats(days=28,chem.name="bisphenol a", dose=20,output.units="mg/L")
# Same study in a mouse:
calc_stats(days=28,chem.name="bisphenol a", dose=20,species="mouse",default.to.human=T)
```

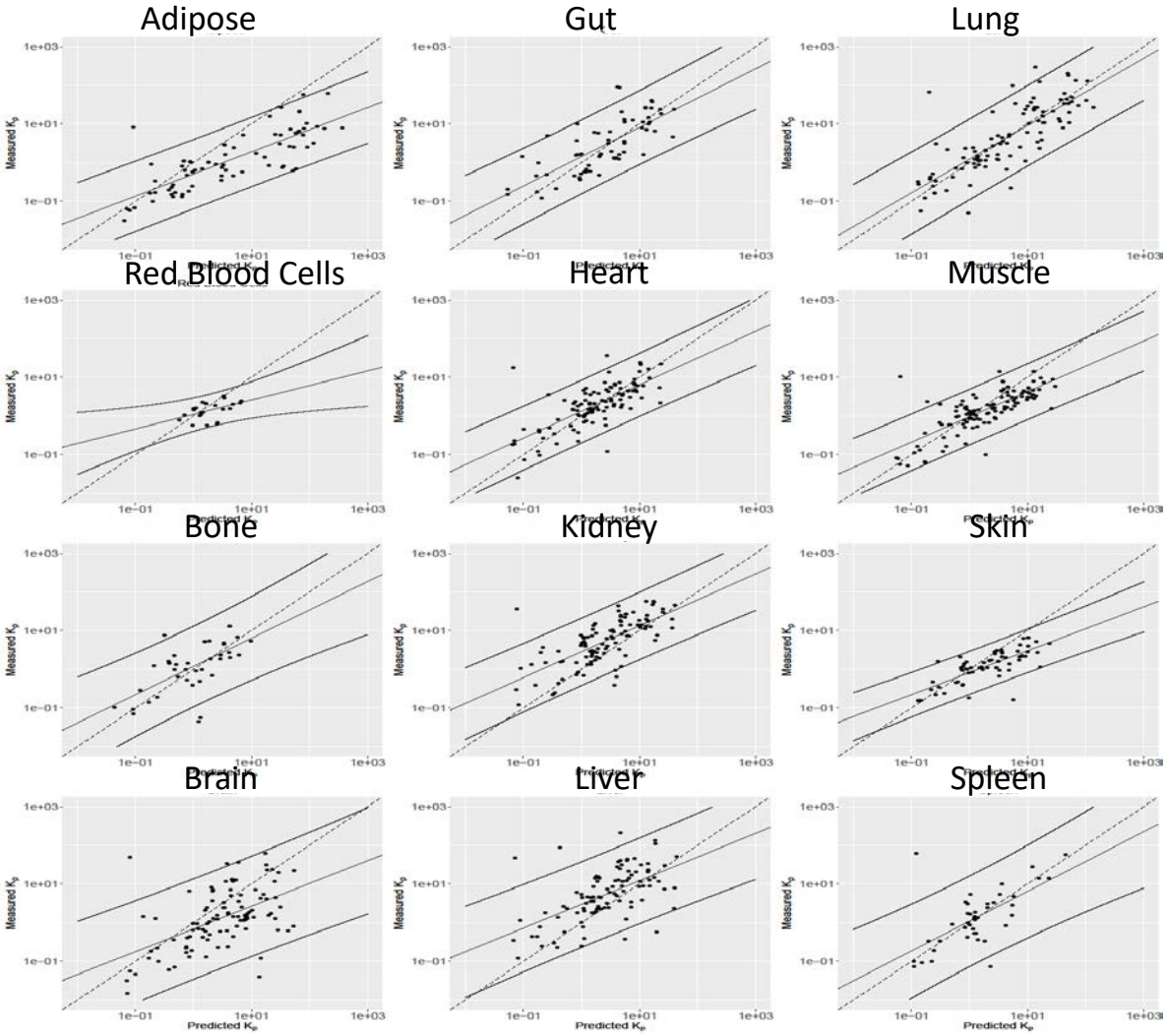
Predicting Partition Coefficients



- Analyzed literature measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
  - Mostly pharmaceuticals
- Calibrating *in silico* predictors (Schmitt, 2008) to actual performance
- Evaluated with human measured volumes of distribution for 498 chemicals from Obach (2008)
  - All pharmaceuticals

Pearce et al. (2017b)

Predicting Partition Coefficients



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  - All pharmaceuticals

Pearce et al. (2017b)

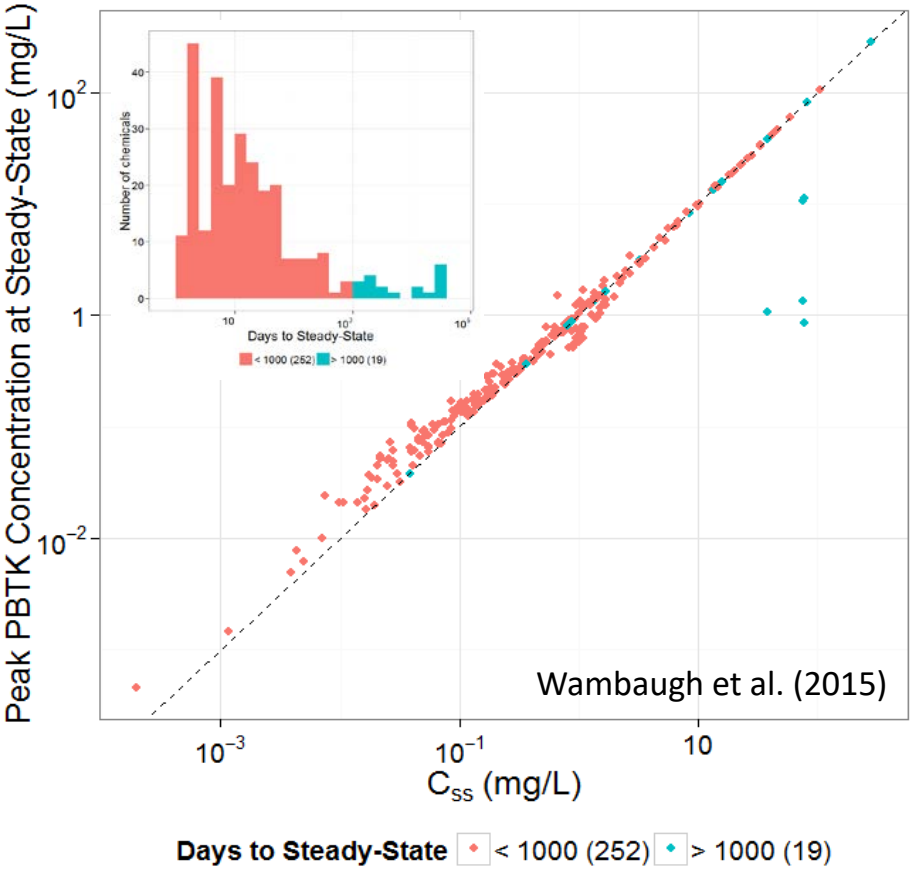
Using the PBPK Solver Directly

```
library(httk)
solve_pbtk(chem.name="bisphenol a")
Human values returned in uM units.
AUC is area under plasma concentration curve in uM * days units with Rblood2plasma = 13.829 .
```

	time	Agutlumen	Cart	Cven	Clung	Cgut	Cliver	Ckidney	Crest	Ametabolized	Atubules	Cplasma	AUC
[1,]	0.00000000	3.066275e+02	0.0000000	0.0000000	0.0000000	0.0000000	0.000000	0.000000	0.0000000	0.0000000	0.000000000	0.00000000	0.0000000000
[2,]	0.01041667	2.388017e+02	0.5991529	0.6287457	1.3199744	21.5143390	16.400297	3.233837	0.1914032	0.6152291	0.001766711	0.04546572	0.0002027523
[3,]	0.02083333	1.859790e+02	1.0004073	1.0083651	2.1406984	21.2910531	23.929492	5.969930	0.8381364	2.3122408	0.009141183	0.07291668	0.0008494912
[4,]	0.03125000	1.448406e+02	1.0588194	1.0574935	2.2507541	18.6383943	23.805194	6.461686	1.6078696	4.2587907	0.018747032	0.07646924	0.0016399193
[5,]	0.04166667	1.128020e+02	0.9900774	0.9858431	2.1000346	15.6437008	21.093573	6.086786	2.3205218	6.0701074	0.028321968	0.07128808	0.0024132951
[6,]	0.05208333	8.785027e+01	0.8881710	0.8835210	1.8825725	12.9223287	17.876882	5.473438	2.9227548	7.6352470	0.037100155	0.06388898	0.0031178197
[7,]	0.06250000	6.841785e+01	0.7883695	0.7841762	1.6709261	10.6387106	14.905516	4.859989	3.4111465	8.9492093	0.044931086	0.05670518	0.0037452376
[8,]	0.07291667	5.328387e+01	0.7019889	0.6984803	1.4881848	8.7907797	12.394544	4.324754	3.7991362	10.0424589	0.051886143	0.05050836	0.0043026722
[9,]	0.08333333	4.149753e+01	0.6310281	0.6281916	1.3382326	7.3221169	10.355693	3.883444	4.1039821	10.9532118	0.058100464	0.04542565	0.0048013867
[10,]	0.09375000	3.231830e+01	0.5741708	0.5719161	1.2181499	6.1656716	8.732407	3.529201	4.3419895	11.7173422	0.063712849	0.04135627	0.0052525642
[11,]	0.10416667	2.516952e+01	0.5291804	0.5274035	1.1231570	5.2594857	7.452953	3.248636	4.5270631	12.3653625	0.068845520	0.03813749	0.0056659289
[12,]	0.11458333	1.960204e+01	0.4938045	0.4924101	1.0484744	4.5511975	6.449790	3.027926	4.6705414	12.9221223	0.073599630	0.03560705	0.0060494826
[13,]	0.12500000	1.526609e+01	0.4660733	0.4649812	0.9899344	3.9982940	5.665391	2.854874	4.7814699	13.4074338	0.078056481	0.03362362	0.0064096387
[14,]	0.13541667	1.188924e+01	0.4443620	0.4435072	0.9441034	3.5669375	5.052878	2.719379	4.8669831	13.8369184	0.082280440	0.03207080	0.0067514674
[15,]	0.14583333	9.259350e+00	0.4273671	0.4266978	0.9082280	3.2304670	4.574870	2.613319	4.9326758	14.2228237	0.086322084	0.03085528	0.0070789492
[16,]	0.15625000	7.211189e+00	0.4140571	0.4135327	0.8801305	2.9679880	4.201883	2.530261	4.9829214	14.5747234	0.090221004	0.02990328	0.0073951988
[17,]	0.16666667	5.616079e+00	0.4036218	0.4032104	0.8581008	2.7631742	3.910801	2.465151	5.0211325	14.9000872	0.094008099	0.02915686	0.0077026468
[18,]	0.17708333	4.373808e+00	0.3954277	0.3951043	0.8408012	2.6032874	3.683555	2.414033	5.0499698	15.2047384	0.097707470	0.02857070	0.0080031886
[19,]	0.18750000	3.406325e+00	0.3889798	0.3887250	0.8271873	2.4783968	3.506044	2.373818	5.0715067	15.4932160	0.101337911	0.02810940	0.0082983022
[20,]	0.19791667	2.652848e+00	0.3838923	0.3836909	0.8164447	2.3807648	3.367276	2.342097	5.0873584	15.7690549	0.104914056	0.02774538	0.0085891383
[21,]	0.20833333	2.066041e+00	0.3798646	0.3797048	0.8079387	2.3043633	3.258686	2.316992	5.0987829	16.0350089	0.108447302	0.02745713	0.0088765933
[22,]	0.21875000	1.609034e+00	0.3766622	0.3765349	0.8011748	2.2444970	3.173600	2.297042	5.1067604	16.2932235	0.111946540	0.02722791	0.0091613663
[23,]	0.22916667	1.253117e+00	0.3741028	0.3740007	0.7957679	2.1975087	3.106820	2.281105	5.1120540	16.5453689	0.115418684	0.02704466	0.0094440009

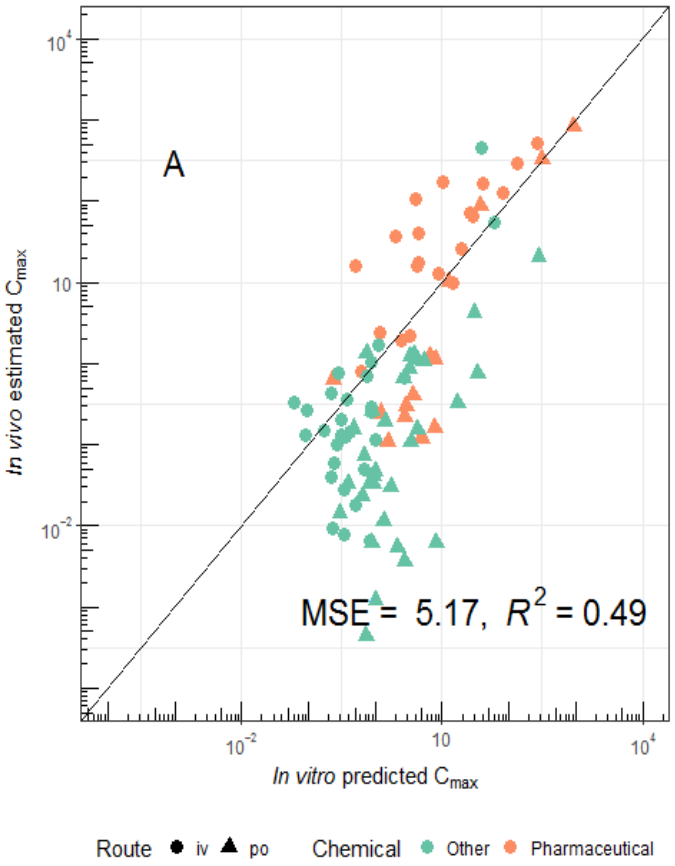
Evaluation of Peak Concentration vs. C_{ss}

- Peak serum concentrations from the HT-PBTK model are compared against the steady-state concentration predicted by the three compartment model for a constant infusion exposure (as in Wetmore *et al.* 2012)
- The dashed, identity (1:1) line indicates that for most compounds the peak concentrations are very similar to C_{ss}



Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data

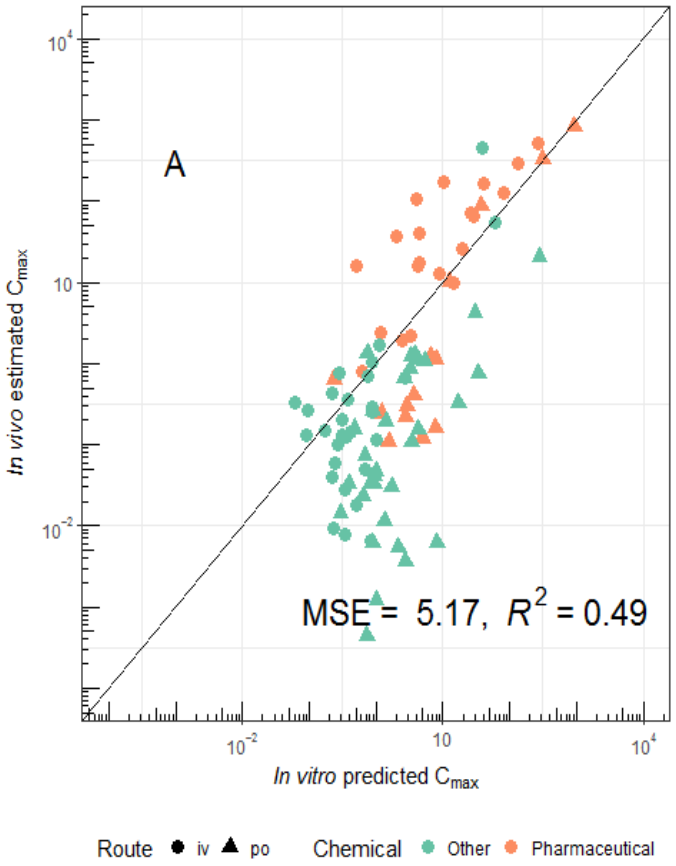
- PBTK predictions can be made for maximum plasma concentration (C_{max}) and for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat

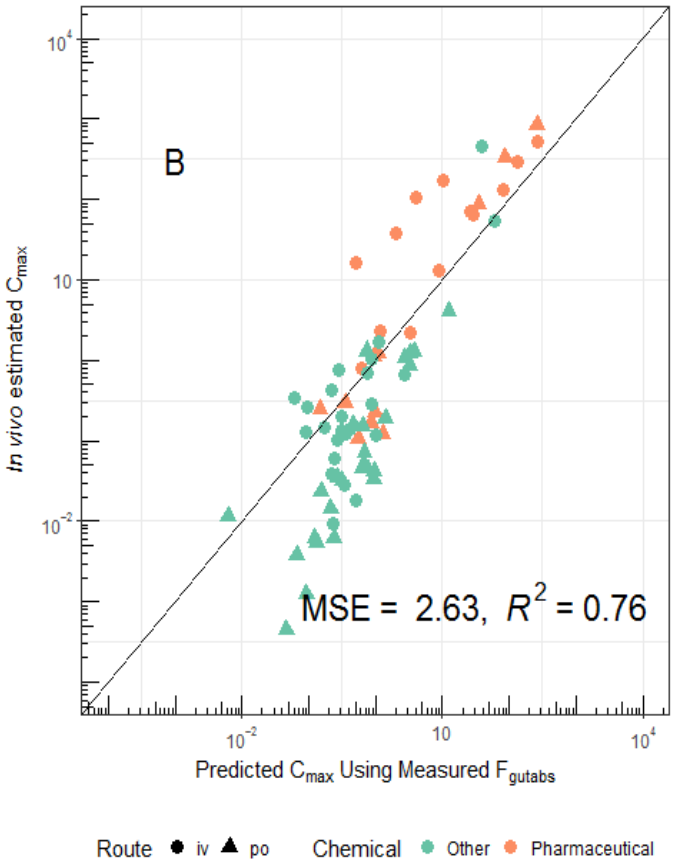


Wambaugh et al., submitted

Evaluating *In Vitro* PBTK Predictions with *In Vivo* Data

- PBTK predictions can be made for maximum plasma concentration (C_{max}) and for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat
- Inclusion of oral bioavailability data improves predictions ("httk" assumes default of 100%)





Wambaugh et al., submitted

Comparison Between HT-PBTK and Chemical Specific PBTK

- We compared a chemical-specific human PBTK model for bisphenol A (Yang et al., 2015) to the HTTK generic PBTK model

- The fitted PBTK model from Yang et al. (2015) and the httk models yielded similar time-plasma concentration curves in the prediction of human *in vivo* data from Thayer et al. (2015)

- We assessed average-fold error (AFE) (the average quotient of the measured and predicted concentrations when the dividend is larger than the divisor)
  - The fitted model (Yang et al., 2015) performed the best, with AFE 1.4
  - However, the generic PBTK model had an AFE of 3.3

Work by Risa Sayre and Robert Pearce

What Can You Do with HTTK?

- Public, open-source set of models and data that have been published in peer-reviewed scientific journals
- Allows PBTK modeling
- Allows conversion of *in vitro* concentration to *in vivo* doses
- A peer-reviewed paper in the Journal of Statistical software provides a how-to guide (Pearce et al., 2017a)
- You can add new chemical information to library and analyze with package tools
- You can use specific demographics from modern U.S. population in the population simulator
  - Gender, age, weight, ethnicity, renal function

For risk assessors, in particular:

- You can load specific (older) versions of the package
- You can control the built-in random number generator to reproduce the same random sequence (function set.seed())

HTTK Limitations (from Ring et al., 2017)

- Oral absorption
  - 100% assumed, but may be very different
  - *In silico* models not necessarily appropriate for environmental chemicals
- Hepatic Clearance (CL_{int})
  - Ten donor pool in suspension for 2-4 h misses variability and low turnover compounds
  - Isozyme abundances and activity: varies with age, ethnicity (at least) (Yasuda et al. 2008, Howgate et al. 2006, Johnson et al. 2006)
  - Parent chemical depletion only
- Isozyme-specific data & modeling (Wetmore et al. 2014)
  - Isozyme-specific metabolism assays not HT
  - *In silico* predictions of isozyme-specific metabolism? Not easy!
    - Existing data is mostly for pharmaceuticals
- Plasma binding assay (F_{up})
  - Assay often fails due to analytical chemistry sensitivity (Wetmore et al., 2012)
  - Plasma protein concentration variability (Johnson et al. 2006, Israili et al. 2001)
  - Albumin or AAG binding? (Routledge 1986)

Version history for "httk"

The publicly available R package contains code and data that has been part of peer-reviewed publications (Old versions are archived)

- Version 1.1 accompanied "Toxicokinetic Triage for Environmental Chemicals" Wambaugh et al. (2015) Tox. Sci.
- Version 1.2 accompanied submission of "httk: R Package for High-Throughput Toxicokinetics" Pearce et al., Journal of Statistical Software (2017a)
- Version 1.3 accompanied "Incorporating High-Throughput Exposure Predictions with Dosimetry-Adjusted *In Vitro* Bioactivity to Inform Chemical Toxicity Testing" Wetmore et al., Toxicological Sciences (2015).
- Version 1.4 addressed comments for revision of Pearce et al., Journal of Statistical Software (2017)
- Version 1.5 accompanied "Identifying populations sensitive to environmental chemicals by simulating toxicokinetic variability," Ring et al. Environment International (2017)
- Version 1.6 accompanied "Evaluation and Calibration of High-Throughput Predictions of Chemical Distribution to Tissues," Pearce et al. (2017b) submission to Journal of Pharmacokinetics and Pharmacodynamics
- Version 1.7 accompanied publication of Pearce et al., Journal of Statistical Software (2017a)

- Subsequent version numbers will be assigned as papers are accepted on:
  - New in vivo data (Wambaugh et al., submitted)
  - In silico HTTK parameter predictions (Sipes et al., 2017)

Lead programmer Robert Pearce

United States Environmental Protection Agency

Summary

- Toxicokinetics (TK) provides a bridge between HTS and HTE by predicting tissue concentrations due to exposure
- High Throughput (HTTK) methods developed for pharmaceuticals have been adapted to environmental testing
- A primary application of HTTK is “Reverse Dosimetry” or RTK
  - Can infer daily doses that produce plasma concentrations equivalent to the bioactive concentrations,
  - **But:** We must consider “domain of applicability”
- New R package “httk” freely available on CRAN allows statistical analyses to identify strengths and weaknesses
  - All HTTK models and data made public upon peer-reviewed publication
- Includes one compartment, three compartment (e.g., Wetmore et al.) and generic PBTK model
- New bioavailability (CACO2) data being collected and analyzed

United States Environmental Protection Agency

Chemical Safety for Sustainability (CSS) Research Program

Rapid Exposure and Dosimetry (RED) Project

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